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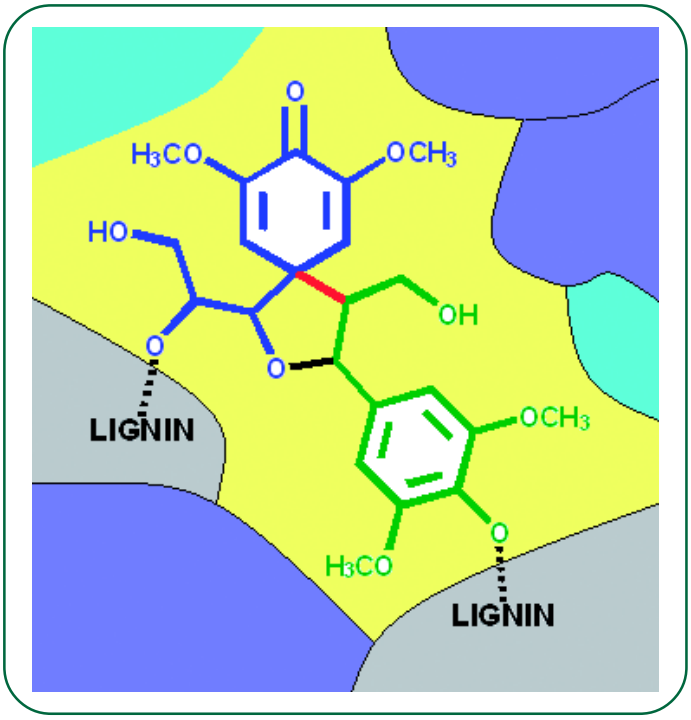

Harri Setälä

Regio- and stereoselectivity of oxidative coupling reactions of phenols

Spirodienones as construction units in lignin

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Spirodienones as construction units in lignin

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ACADEMIC DISSERTATION

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Setälä, Harri. Regio- and stereoselectivity of oxidative coupling reactions of phenols. Spirodienones as construction units in lignin [Fenolien hapettavan kytkentäreaktion regio- ja stereoselektiivisyys. Spirodienonit ligniinin rakenneyksiköinä]. Espoo 2008. VTT Publications 689. 104 p. + app. 38 p.

Keywords regioselectivity, stereoselectivity, oxidative coupling reactions, phenols, spirodienones, lignans, dilignols, dehydrodimerization, peroxidases, chirality, pH, catalysts

Abstract

Dimeric phenolic compounds – lignans and dilignols – form in the so-called oxidative coupling reaction of phenols. Enzymes such as peroxidases and laccases catalyze the reaction using hydrogen peroxide or oxygen, respectively, as oxidant generating phenoxy radicals which couple together according to certain rules. In this thesis, the effects of the structures of starting materials – monolignols – and the effects of reaction conditions such as pH and solvent system on this coupling mechanism and on its regio- and stereoselectivity have been studied.

After the primary coupling of two phenoxy radicals a very reactive quinone methide intermediate is formed. This intermediate reacts quickly with a suitable nucleophile which can be, for example, an intramolecular hydroxyl group or another nucleophile such as water, methanol, or a phenolic compound in the reaction system. This reaction is catalyzed by acids. After the nucleophilic addition to the quinone methide, other hydrolytic reactions, rearrangements, and elimination reactions occur, leading finally to stable dimeric structures called lignans or dilignols. Similar reactions occur also in the so-called lignification process when monolignol (or dilignol) reacts with the growing lignin polymer. New kinds of structures have been observed in this thesis. The dimeric compounds with a so-called spirodienone structure have been observed to form both in the dehydrodimerization of methyl sinapate and in the β-1-type cross-coupling reaction of two different monolignols. This β-1-type dilignol with a spirodienone structure was the first synthesized and published dilignol model compound, and at present, it has been observed to exist as a fundamental construction unit in lignins.

The enantioselectivity of the oxidative coupling reaction was also studied for obtaining enantiopure lignans and dilignols. A rather good enantioselectivity was obtained in the oxidative coupling reaction of two monolignols with chiral auxiliary substituents using peroxidase/H_2O_2 as an oxidation system. This observation was published as one of the first enantioselective oxidative coupling reaction of phenols. Pure enantiomers of lignans were also obtained by using chiral cryogenic chromatography as a chiral resolution technique. This technique was shown to be an alternative route to obtain enantiopure lignans or lignin model compounds in a preparative scale.

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Avainsanat regioselectivity, stereoselectivity, oxidative coupling reactions, phenols, spirodienones, lignans, dilignols, dehydrodimerization, peroxidases, chirality, pH, catalysts

Tiivistelmä

Dimeeriset lignaanit ja dilignolit muodostuvat ns. fenolien hapettavassa kytkentäreaktiossa, jossa fenolisista monolignoleista syntyvät fenoksiradikaalit kytkeytyvät toisiinsa tiettyjen lainalaisuuksien mukaisesti. Reaktiota katalysoivat entsyymit, kuten peroksidaasit ja lakkaasit, sopivan hapettimen – joko vetyperoksidin tai hapen – läsnä ollessa. Tässä väitöskirjassa käsitellään näiden kytkeytymisten seurauksena syntyvien primääristen rakenteiden ja sitä kautta syntyvien dimeeristen yhdisteiden syntymekanismeja ja niihin vaikuttavia tekijöitä, kuten sitä, mitkä lähtöaineen rakenteesta johtuvat stereoelektroniset syyt johtavat erilaisten dimeeristen rakenteiden syntyyn; ja mikä on reaktio-olosuhteiden vaikutus näiden rakenteiden syntyyn. Tässä väitöskirjassa on tutkittu kuuden erilaisen monolignolin rakenteen sekä liuotinsysteemin ja pH:n vaikutusta; ja myös jonkin verran katalyytin sekä hapettimen vaikutusta reaktioiden regio- ja stereoselektiivisyyteen.

Hapettavan kytkentäreaktion jälkeen tapahtuvat sekundääriset reaktiot, kuten nukleofiilinen additio kinonimetidivälituotteeseen ja sitä seuraavat erilaiset hydrolyyttiset reaktiot, toisiintumiset ja eliminoitumisreaktiot, johtavat lopulta stabiileisiin dimeerisiin rakenteisiin. Näihin reaktiovaiheisiin vaikuttavia tekijöitä on myös käsitelty tässä väitöskirjassa. Kinonimetidi on syntyvän kytkentäreaktion tuote, välituote, joka on hyvin reaktiivinen (vaikkakin voi olla tietyissä olosuhteissa melko pysyvä) ja reagoi nukleofiilien kanssa joko molekyylien välisissä reaktioissa (vesi, fenolinen tai alifaattinen hydroksyyliryhmä, tiolit yms.) tai molekyylin sisäisesti esim. tarjolla olevan hydroksyyliryhmän kanssa synnyttäen mm. erilaisia rengasrakenteita (furaanit, bentsofuraanit). Nämä rakenteet ovat melko pysyviä ja yleisiä eristetyissä lignaaneissa ja ligniineissä. Kuitenkin jotkin niistä voivat olla myös välituotteita muiden lignaanien muodostumisreitissä ja myös mahdollisia reittejä tiettyjen ligniinissä esiintyvien rakenneosien muodostumiselle. Eräs tällainen

välituotetyyppi ovat ns. spirodienonirakenteiset yhdisteet, joita esiintyy luonnossa stabiileina rakenteina lignaaneissa ja ligniinissä. Spirodienonirakenteinen dimeeri kuitenkin reagoi melko helposti mm. happamissa olosuhteissa toisiintumalla eri rakenteeksi. Spirodienonirakenteet selittävät osaltaan ligniinien ns. β-1-rakenteiden syntymismekanismeja. Yleisesti ottaen varsinaisen hapettavan kytkentäreaktion jälkeiset sekundääriset reaktiot voivat olla hyvin monimutkaisia ja johtaa suureen määrään rakenteellisesti hyvin erilaisia dimeerejä – lignaaneja. Lähtöaineiden rakenteen ja reaktiota katalysoivan entsyymi-hapetinsysteemin lisäksi pH-vaikutus, liuotinsysteemi, muiden nukleofiilisten reagoivien aineiden vaikutus (nukleofiilisyys, konsentraatiot); ja intra- vs. intermolekulaarisen reaktion nopeus välituotteen stabiloitumisessa lopputuotteeksi ovat tärkeitä reaktioparametreja.

Polymeerisen ligniinimolekyylin syntyessä kytkeytymisreaktion lainalaisuudet ovat osin toisenlaisia, koska tässä reaktiotyypissä – polymeroitumisessa – kasvava ligniinimolekyyli reagoi monomeerisen (tai dimeerisen) fenolisen yhdisteen, monolignolin, kanssa. Vallitseva selitys lignifikaatiosta, ligniinin syntymisestä, perustuu teoriaan, jonka mukaan tietyistä käytettävissä olevista monomeerisista yhdisteistä, monolignoleista, syntyy tiettyjen kombinatoriaalisen kemian lainalaisuuksien mukaan erilaisia ligniinin perusrakenneosia ilman esimerkiksi entsyymin ohjaavaa vaikutusta. Syntyvien rakenteiden keskinäinen suhde ligniinissä perustuu pikemminkin reagoivien monolignolien rakenne-eroavaisuuksiin (hapetuspotentiaalit, stereoelektroniset tekijät), konsentraatioihin ja syöttönopeuteen ligniinipolymeerin kasvaessa hapettavassa kytkentäreaktiossa; sekä erilaisten reaktioolosuhteiden vaikutukseen. Tässä väitöskirjatyössä syntetisoitu β-1-ristikytkentämekanismilla syntynyt dimeeri on laatuaan ensimmäinen kokeellisesti valmistettu spirodienonirakenteinen dilignoliyhdiste. Rakenteen on myöhemmin todennettu esiintyvän yleisesti yhtenä ligniinien perusrakenneosana. Väitöskirjassa on valmistettu myös muita spirodienonityyppisiä dimeerejä.

Lisäksi väitöskirjassa on tutkittu monolignoliin liitetyn kiraalisen substituentin vaikutusta hapettavan kytkentäreaktion enantioselektiivisyyteen. Menetelmällä pystyttiin valmistamaan dimeerisiä rakenteita hyvällä enantioselektiivisyydellä. Julkaisu on eräs ensimmäisistä maailmassa. Puhtaita enantiomeereja voidaan valmistaa myös käyttäen ns. kiraalisia resoluutiotekniikoita. Tässä työssä tutkittiin ns. kiraalisen kromatografian käyttöä puhtaiden enantiomeerien valmistamiseksi raseemisista lignaaneista.

Preface

The experimental work for this thesis was carried out at the Laboratory of Organic Chemistry of the University of Helsinki. I am grateful to Professor Tapio Hase and Professor Gösta Brunow for placing the excellent research facilities of the laboratory at my disposal.

I am deeply grateful to my supervisor, Professor Gösta Brunow, for his encouragement and continuous support and patience during the many years of this work as he has waited for the publications of the results and at last for the completion of this thesis.

I would like to express my thanks to Dr. Jorma Matikainen for running the mass spectra, to Seppo Kaltia and Prof. Ilkka Kilpeläinen for co-operation in the field of NMR studies, and to all my collegues at the laboratory of organic chemistry and all over the chemistry department of Helsinki University.

The “Thursty Club” has been a very important group of my collegues, to mentition a few, Pekka Pietikäinen, Jussi Sipilä, Vesa Nevalainen, Merja Toikka. This club met on Thursdays to drink good beers and to discuss the meaning of life and science.

I wish also to thank my family: my wife Tiina and my children Sini and Suvi, and also my father Aimo and mother Helvi, and of course my brothers Vesa and Jukka, and my sister Eija. I believe that all of them never gave up on me.

Financial support was from Tekes – the Finnish Funding Agency for Technology and Innovation.

List of original publications

This thesis consists of the following papers, which will be referred to in the text by their Roman numerals (Papers I–VI)

I. Chioccara, F.; Poli, S.; Rindone, B.; Pilati, T.; Brunow, G.; Pietikäinen, P.; Setälä, H., Regio- and diastereo-selective synthesis of dimeric lignans using oxidative coupling. *Acta Chem. Scand*. **1993**, *47*, 610–616.

II. Setälä, H.; Pajunen, A.; Kilpeläinen, I.; Brunow, G., Horse radish peroxidase-catalyzed oxidative coupling of methyl sinapate to give diastereoisomeric spiro dimers. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1163–1165.

III. Pajunen, A.; Brunow, G.; Setälä, H., Dimethyl 1-(4-acetoxy-3,5-dimethoxy-phenyl)-4,7,9-trimethoxy-8-oxospiro[4.5]-deca-6,9-diene-*trans*-2,3-dicarboxylate acetone solvate. *Acta Cryst.* **1994**, **C***50*, 1823–1825.

IV. Bolzacchini, E.; Brunow, G.; Meinardi, S.; Orlandi, M.; Rindone, B.; Rummakko, P.; Setälä, H., Enantioselective synthesis of a benzofuranic neolignan by oxidative coupling. *Tetrahedron Lett*. **1998**, *39*, 3291–3294.

V. Setälä, H.; Pajunen, A.; Rummakko, P.; Sipilä, J.; Brunow, G., A novel type of spiro compound formed by oxidative cross coupling of methyl sinapate with a syringyl lignin model compound. A model system for the *β*-1 pathway in lignin biosynthesis. *J. Chem. Soc., Perkin Trans. 1* **1999**, 461–464.

VI. Alkio, M.; Aaltonen, O.; Setälä, H., Cryogenic chiral chromatography for rapid resolution of drug candidates. *Org. Proc. Res. Develop.* **2005**, *9*, 782–786.

Contents

List of symbols and abbreviations

AcOH	Acetic acid
CAL	Coniferyl alcohol
CPO	Chloroperoxidase
CSP	Chiral solid phase
DFRC	Derivatization followed by reductive cleavage
DFT	Density functional theory
DHP	Dehydrogenation polymer
EPR	Electron paramagnetic resonance
Et	Ethyl
EtOH	Ethanol
FA	Ferulic acid
HMPA	Hexamethylphosphoramide
HMQC	Heteronuclear multiple quantum coherence
HOHAHA	Homonuclear Hartman Hahn
HRP	Horseradish peroxidase
IEG	Isoeugenol
IPA	Isopropanol
LiP	Ligninperoxidase
LPO	Lactoperoxidase

MD	Molecular dynamic
Me	Methyl
MeFA	Methyl ferulate
MeOH	Methanol
MeSA	Methyl sinapate
Mn(III)TPP	Tetraphenylporhyrinatomanganase
MnP	Manganase-dependent peroxidase
MS	Mass spectroscopy
NADP	Nicotinamide adenine dinucleotide phosphate
NMR	Nuclear magnetic resonance
oHBA	*o*-Hydroxybenzyl alcohol
QM	Quinone methide
SA	Sinapic acid
SFC	Supercritical fluid chromatography
SOMO	Single occupied molecular orbital
THF	Tetrahydrofuran
TsOH	*p*-Toluenesulfonic acid
ZT	Zutropf(verfahren)

1. Introduction

Both the dimeric lignans and dilignols, and the structural units in the lignin are formed in the so-called oxidative coupling reaction of phenols. Erdtman [1] proposed already in 1933 the main features for the formation of a dimer in the oxidative coupling reaction of phenolic compounds. He used isoeugenol as the model compound. Freudenberg [2] developed this idea by using coniferyl alcohol as the lignin precursor and the so-called dehydrogenation polymers (DHPs) were obtained by using enzymes or inorganic oxidants. After those days lignin chemistry and research have developed on many fronts including the findings of phenylpropanoid pathways and other biosynthetic routes to lignans and lignin. These results have been reviewed, for instance, in the book of Lewis and Sarkanen. [3] The most important and relevant results published in the field of lignan and lignin research from the beginning of the 1980's to 2008 in relation to this thesis are reviewed and referred to in Chapter 2 as an introduction to the results and discussion in Chapter 4.

This thesis and the published results (Papers I–VI) are just a small part in the great puzzle of lignan and lignin research but some important new information and pieces of that puzzle have been found. The effects of the structure of monolignols and some reaction parameters of the reaction conditions on the formation of dimeric dilignols (lignans) have been studied by using some selected monolignols as model compounds. First, both the effect of the structure of three monolignols – isoeugenol (**1**), methyl ferulate (**2**) and coniferyl alcohol (**3**) – and the effect of the pH and organic cosolvents on the amounts and types of dilignols have been studied (Paper I). These findings led onto the next studies with methyl sinapate as the starting material and onto the first observations of new spirodienone structures (Papers II and III). More closely related to the lignin chemistry, a new dilignol with a spirodienone structure was obtained in the oxidative β-1-type cross-coupling reaction of two monolignols (Paper V).

Lignans in nature exist usually as pure enantiomers or as enantiomeric mixtures. [4] Many asymmetric synthetic methods are available for preparing pure enantiomers of lignans. Monolignols with chiral auxiliary substituents were used to study the enantiomeric selectivity in the oxidative coupling reaction (Paper IV). These results were published as one of the first observations in the world by using this

kind of a method. Because the enantiomeric selectivity (enantiomeric excess) is not often high enough and a product is a mixture of enantiomers, other separation and purification methods are needed. Chiral resolution using some chromatographical methods were also studied for the production of pure enantiomers of lignans, in preparative scale (Paper VI).

2. Formation and structure of lignans and lignin

Monomeric phenolic compounds such as monolignols are derived from phenylalanine *via* general phenylpropanoid pathways in plants. Monolignols are phenolic compounds with a phenylpropane carbon skeleton. Other monomeric, dimeric, oligomeric, and polymeric phenolic coumpounds such as 1) benzoates and salicylates, 2) coumarins, 3) cinnamics and phenylpropenes, 4) lignans, 5) flavonoids, 6) stilbenes, 7) tannins, and 8) lignin polymers are derived basically from monolignols and/or related phenolic compounds. The phenylpropanoid pathways and other biosynthetic pathways leading to phenolic compounds and the enzymes involved in these pathways are rather well known and reviewed in literature. [5–9] A simplified scheme of the phenylpropanoid pathways is presented in Figure 2. The most interesting compounds and pathways that are a part of this thesis are coloured red. Many other monomeric hydroxycinnamics such as isoeugenol and eugenol are also biosynthetized through phenylpropanoid pathways, see Figure 2. [10]

The transportation and/or rate of diffusion of monolignols from cells where they are biosynthetized to cell walls or other places where they are needed and used, is the next step before the oxidative coupling reaction and/or lignification processes. They are transported as their glycosides such as coniferin (Figure 1) which has been observed to be present in the lignifying tissue of conifers. [2] Coniferin is hydrolyzed by β-glucosidases to coniferyl alcohol and a mono-saccharide. [2, 11, 12] Many studies, for example, using radio- or ^{13}C-labeled coniferin have shown that coniferin is incorporated in lignans or in the lignin of the cell walls. [13–16]

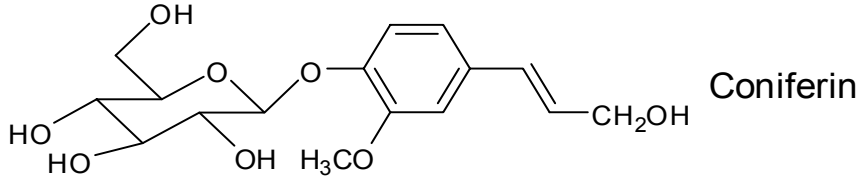


Figure 1. Coniferyl alcohol 4-O-β-D-glucoside, coniferin.

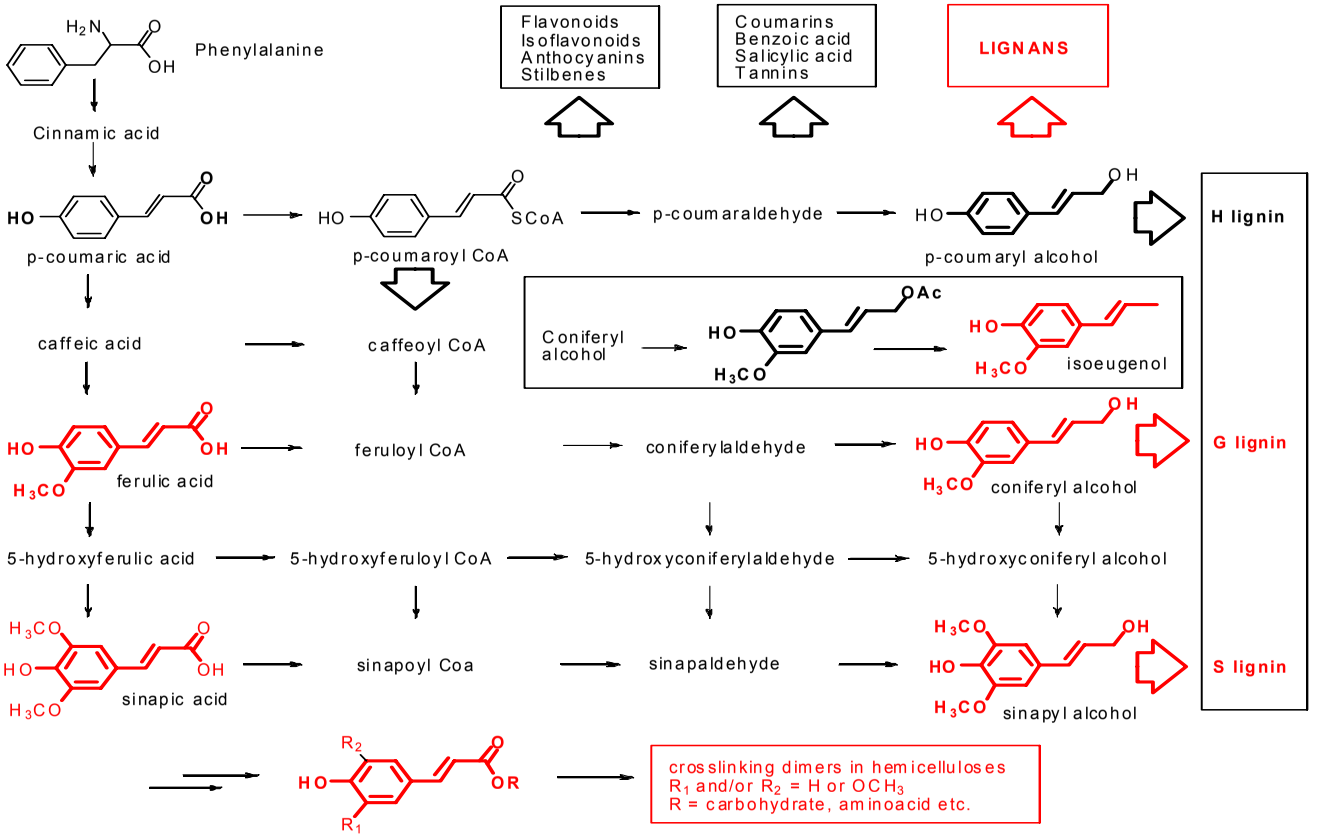


Figure 2. Biosynthesis of phenolics through phenylpropanoid pathways.

2.1 Lignans and dilignols

Lignans, neolignans, norlignans, and other phenolic compounds are widely distributed in vascular plants. Phenylpropanoid dimers often closely related to lignans and neolignans are also called dilignols, but this term is used mostly when speaking about lignin chemistry and lignin precursors. Many lignans are reported to be optically active existing in plants as pure enantiomers or optically active mixtures of enantiomer pairs or racemic mixtures. [17] The origin of chirality of lignans will be discussed in more detail in Section 2.5.

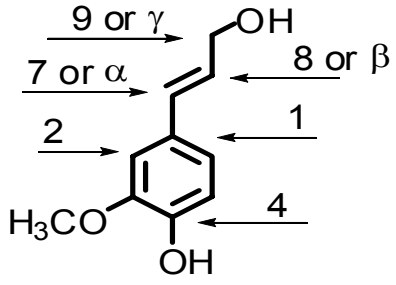


Figure 3. Two common ways of labelling the carbon atoms in monolignols.

Most lignans and dilignols are biosynthesized from monomeric phenolic phenylpropanoid compounds such as coniferyl alcohol, sinapyl alcohol, ferulic acid, or caffeic acid. They are usually dimeric compounds (dilignols) but, for example, trimeric lignans also exist. [18] Flavonolignans [19] and alkaloids [20] with a dimeric phenolic skeleton and with amine functionality, and so-called norlignans [21] have also been separated and identified in plants. Some examples are presented in Figures 4 and 5. There is only a relatively small number of phenylpropanoid interunit primary linkages that are used to divide lignans into sub-groups: 8-8' (β-β), 8-1'(β-1), 8-5' (β-5), 8-O-4' (β-O-4), 5-5', 4-O-5 etc. This system of nomenclature is based on the structural features and on the way the monomers are coupled: dimers with 8-8' coupling (or β-β) are lignans, dimers with 8-5' (β-5) or 8-O-4' (β-O-4) coupling are neolignans, etc. (see IUPAC Nomenclature of Lignans and Neolignans). [22] This nomenclature is used in lignan chemistry. [21, 23] The coding system of carbon atoms in monolignols presented in parenthesis is widely used in lignin chemistry [24, 25], and also used in this thesis (see Figure 3). All coupling combinations mentioned above exist in lignin. The β-β' and β-5 couplings are the structures most

abundant in lignans. The main groups of β-β' lignans, their structures, and some examples of natural lignans are presented in Figure 4. The biosynthesis, biodiversity, and biological function of lignans are reviewed elsewhere. [4, 23, 26]

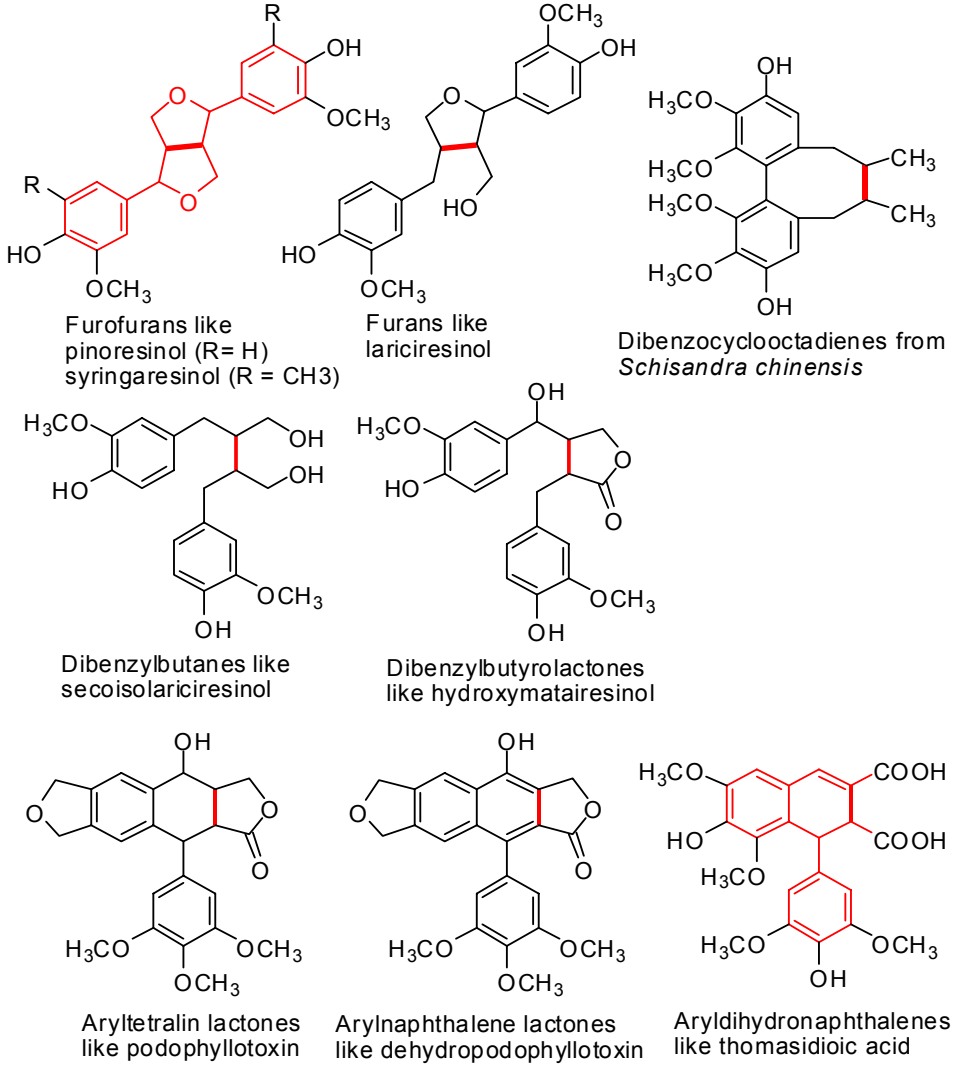
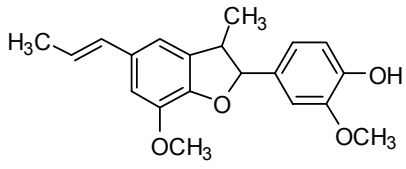


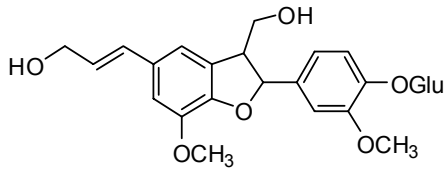
*Figure 4. Main types of lignan groups resulting from β-β coupling of phenoxy radicals. The β-β bond is shown in **red** and **bolded**. The compounds shown in red will be discussed in more detail in this study.*

The two natural isomers of hydroxymatairesinol are the most abundant lignans present in Norway spruce (*P. abies*) and may comprise up to 0.3-% of the dry wood content. [27] Knots (inner branches) may contain even 6–25-% lignans (w/w) with hydroxymatairesinol dominating. [28] Most fir (*Abies*) species contain secoisolariciresinol, lariciresinol, and pinoresinol as the main lignans [29], see Figure 4. Amounts and types of lignans are dependent on wood and plant species and their distribution inside the species (knots, stem, etc.) is reviewed elsewhere. [30, 31] Aryltetralins such as podophyllotoxin extracted from the leaves of American mayapple (*Podophyllum peltatum* L.) are promising anticancer agents and some of their derivatives have already been used as pharmaceuticals. [32] Thomasidioic acid with a similar structure as podophyllotoxin has been isolated from *Ulmus thomasii* heartwood [33] and its methyl ester has been synthetized (Paper II). Dibenzocyclooctadiene lignans are common in *Schisandra chinensis*. [34]

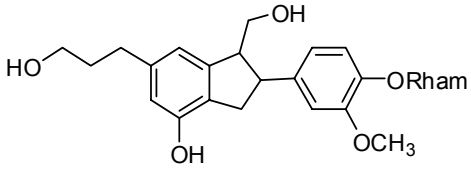
Some examples of the structural diversity of neolignans, norlignans, and related phenolics in plants are presented in Figure 5. Many of them are found – together with lignans and other phenolics – in conifers, monocotyledons, and other trees. [21, 35] Some neolignans isolated from roots of *Krameria gray* [36], or from *Linum usitatissimum* cell cultures [37] in Figure 5 are examples of dihydrobenzofuran-type structures. Lignans and other similar phenolics exist often as their glycosides such as dehydrodiconiferyl alcohol-4-β-D-glycoside from *Linum usitatissimum* [37], or a neolignan rhamnoside from birch leaves [38], but also as free aglycones such as dehydrodiisoeugenol from *Krameria grayi*. [36] Lignans are usually free in trees but glycosides in other plants. Hinokiresinol obtained from suspension-cultured *Cryptomeria japonica* [39], and a spirocyclic sequosempervirin A from the *Sequila sempervirens* plant [40] are examples of norlignans. Some alkaloids such as salutaridine with a spirodienone-like structure have dimeric structure similar to lignans and dilignols. The oxidative coupling reaction step is assumed to be a part of their biosynthesis because of the phenolic functionalities in their structures. [20] Silybin is a so-called flavonolignan isolated from the seeds of *Silybum marianum*. [19] Sesquineolignans with spirodienone structure was observed from Pine (*Pinus sylvestris* L.) bark. [18]



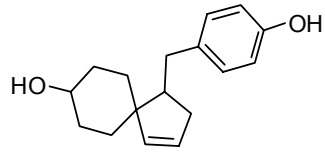
A neolignan from roots of *Krameria grayi*



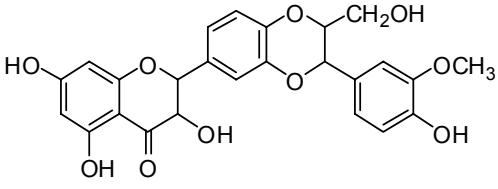
A neolignan glucoside from *Linum usitatissimum* cell cultures



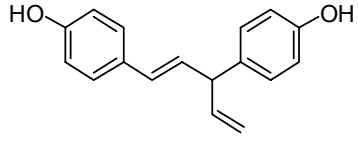
A neolignan rhamnoside from birch leaves (*Betula platyphylla)*



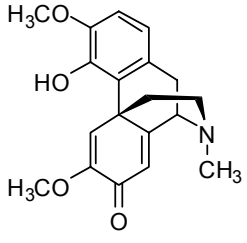
Sequosempervirin-A, a novel spirocyclic compound from *Sequoia sempervirens*



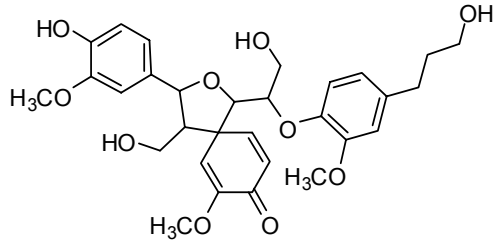
A flavonolignan silybin from the seeds of *Silybum marianum*



Hinokiresinol (norlignan) from suspension-cultured *Cryptomeria japonica*



Salutaridine (alkaloid) from *Antizoma angustifolia* (Menispermaceae)



Sesquineolignan (spirodienone) from Pine (*Pinus sylvestris* L.) bark

Figure 5. Some examples of other dimeric phenolic compounds in plants: dihydrobenzofuran (phenylcoumaran) neolignans, spirocyclic sequosempervirin A and hinokiresinol norlignans, flavonolignan, and alkaloid salutaridine with spirodienone-like structure. The last example is a trimeric neolignan also with spirodienone structure.

2.2 Oxidative coupling reaction of monolignols: dehydrodimerization

Dehydrodimerization is based on the oxidative coupling reaction of phenols where two phenoxy radicals are first generated in an one-electron oxidation reaction by an oxidant system such as peroxidase/H_2O_2 where peroxidase is a catalyzing enzyme and H_2O_2 is an oxidant. Inorganic oxidants such as Ag_2O, hexacyanoferrates or $FeCl_3$ can also be used to generate phenoxyradicals (Figure 6). Two phenoxy radicals are then coupled to form quinone methide intermediates which further react with suitable nucleophiles in intra- or intermolecular reactions. [1, 2, 41] Furthermore, other hydrolytic reactions, eliminations, and/or rearrangements follow yielding the stable end-products, dilignols and lignans. When these two coupling phenoxyradical monomers are not identical, a so-called cross-coupling reaction may take place. Cross-coupling reactions will be discussed in more detail in Section 2.7.

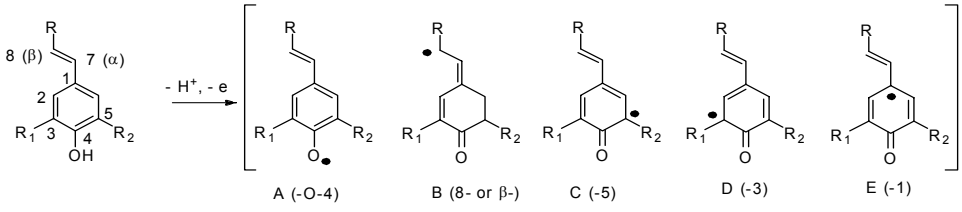


*Figure 6. One-electron oxidation of phenols having a propenyl side chain generating a phenoxy radical (**A**). All the resonance forms (A-E) of a phenoxy radical are presented. The electron spin density of the phenoxy radical is delocalized over the aromatic ring and double bond system giving several positions to react with each other. The oxidative coupling of these positions gives different kinds of primary C-C and C-O bonds and different kinds of lignans.*

In order to achieve a better understanding of what factors and reaction parameters have the greatest effect on this oxidative process from monolignols to dimeric products, the dehydrodimerization and polymerization processes are divided into some basic steps as presented by Shigematsu et al. [42] for the overall polymerization process of DHP (dehydrogenative polymer). Transportation and diffusion of a monolignol and an enzyme in the polysaccharide matrix is the first step in this process but not further discussed in this thesis. Secondly, an oxidant (H_2O_2 or O_2) penetrates into the active site of the enzyme forming an

oxidized/activated enzyme which is further able to oxidize a substrate. Penetration of a monolignol into the active site of the enzyme and the formation of monolignol-enzyme complex is the next step. The formation of monolignol-enzyme-complex is related to the specificity and activity of the enzyme. [43] The ratio and availability of monomers in the reaction side of the cell wall together with oxidizing enzymes is an important factor. [44] The relative reactivities of monolignols (redox potentials) are also important factors in cross-coupling reactions in the dehydrogenative polymerization of monolignols to the growing lignin polymer. [45]

Thirdly, the formed phenoxyradical will be coupled to another phenoxyradical forming quinone methide intermediates. The catalyst can direct reactions in many ways, i.e. act as a chiral promoter (with or without any cofactors or dirigent proteins) yielding optically active lignans. Reaction conditions have a very strong effect on the ratio and amounts of coupling products (Paper I). [43]

Fourthly, post-coupling reactions will occur: 1) Reaction of the quinone methide with a suitable nucleophile and 2) hydrolytic reactions, eliminations, and/or rearrangements follow to yield stable end-products.

These reaction parameters are further discussed in the next sections and Chapter 4.

2.2.1 Formation of phenoxy radicals by one-electron oxidation of monolignols and their coupling to dimers

How the two phenoxy radicals are initially coupled, which reaction route is selected, and the ratio of possible dimeric products are all dependent primarily on the stereoelectronic effects related to the structure of the phenoxy radicals. [42, 46, 47] But also the catalyst/oxidant system and reaction conditions can have remarkable effects (see Section 2.4, and results and discussion in Chapter 4).

The possible coupling positions leading to different kinds of C-C or C-O bonds and lignan types and structural components of lignin are presented in Figure 7. The β-β, β-5, and β-O-4 couplings give the most abundant structures in lignans and also in lignin. The 4-O-5'/ (A + C) coupling is possible only if there is no substituent in the C-5 (and/or C-3) position of the aromatic ring, and usually this

coupling occurs when there is no β-coupling possibility in another phenoxy radical forming compound. 5-5' (C+ C) is also possible if the C-5 (and/or C-3) position has no substituent and is most likely to occur if there is no β-radical coupling possibility in either phenoxy radical forming compound. β-1 (8-1') (B+E) coupling is possible if there is no β-radical coupling possibility in another phenoxy radical forming compound and more likely if at the same time the C-3 and (or) C-5 positions in the aromatic ring are blocked.

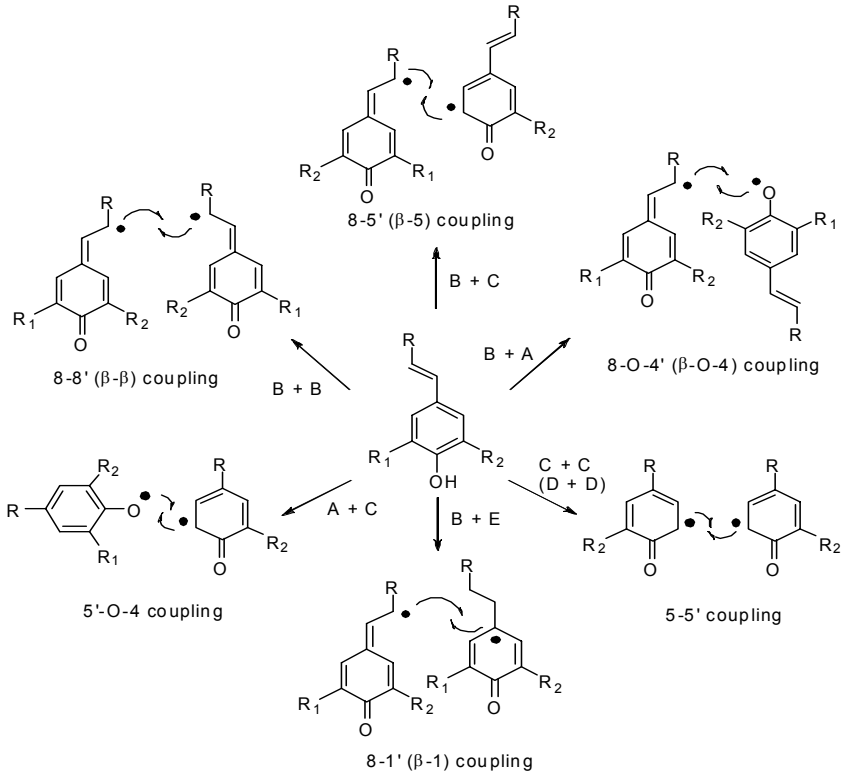


Figure 7. Possible combinations of resonance stabilized phenoxy radicals generated from 4-hydroxycinnamics. For example, β-5 and 5-5' couplings are possible only if there is no substituent in the C-5 (and/or C-3) position of the aromatic ring.

A+A and E+E coupling products have not been observed in lignans and lignin. A+A coupling yields a very unstable peroxy compound. E+E coupling is also theoretically possible but because of a common substitution in this position, it is sterically hindered.

Many theoretical explanations, semi-empirical calculations, and computational methods have been used to determine the factors which could control the formation of various dimeric products, and these results have been compared to experimental data. Houtman [48] simulated the collision of two monolignol molecules. The theoretically feasible linkages of radical coupled intermediates were simulated and their reactivities were compared to the heat of formation by Elder and Ede. [49] The transition state leading to β-O-4 quinone methide intermediate of *p*-coumaryl alcohol was analyzed by semi-empirical molecular orbital calculations by Shigematsu et al. [42] Many kinds of computational and simulation methods have been used. [50, 51] The reactivity of possible coupling positions (C or O atoms) in a phenoxy radical is explained to be dependent on the single-electron spin density at these positions. The spin density is dependent on the substitution in an aromatic ring [51] and on the structure of the C_3-side chain which is usually an unsaturated propenyl chain with a hydroxyl, methyl, or carboxylic acid group at the C_γ-position. [42] Elder and Worley [52] used semi-empirical methods to calculate the spin densities on all atoms in the coniferyl alcohol molecule, but the results did not correlate very well with the experimental data. They looked later also at thermodynamic control as an explanation of the experimental data. However, the heats of formation of the final dilignols did not determine the product distribution. [49] Similar results were obtained later by Durbeej et al. [46, 53] who used density functional theory studies. Houtman [48] suggests that the dimerization of coniferyl alcohol is not under thermodynamic control and that it is unlikely that the main coupling reaction and post-reactions could be reversible. Kinetic arguments were used to explain the product distribution in the case of coniferyl alcohol. Based on the molecular dynamics (MD) results and the experimental results of Terashima and Atalla [54], Houtman [48] has proposed a mechanism by which the solvent environment determines the product distribution of radical-radical coupling reactions. Terashima and Atalla [54] measured the product distribution of dimers and oligomers of coniferyl alcohol in various water/diglyme mixtures and studied pH effects, also. Even a small addition of diglyme (20-%) increased the production of β-O-4 dimer up to approx. 40-%. The results of Houtman [48], and Tereshima and Atalla [54], are compared in Table 1.

Table 1. Product distribution of the oxidative coupling of coniferyl alcohol based on MD simulations [48] and experimental data [54] compared to statistical calculations (random).

(%) of a dimer:	β-O-4	β-5	β-β	Others / oligomers	Ref.
MD simul. (water)	13	56	31	-	[48]
Random	40	40	20	-	[48]
Exp. in water	19	34	27	-	[48]
20-% diglyme, pH 5	40	30	13	17	[54]
60-% diglyme, pH 5	50	32	9	8	[54]
50-% diglyme, pH 4	50	33	12	6	[54]
50-% diglyme, pH 7	29	40	13	17	[54]

Phenoxy radicals are assumed first to form a so-called π-complex. The phenoxy radicals have to be superimposed in a way that enables the maximum overlapping of single-occupied molecular orbitals (SOMO), and at the same time the stereoelectronic repulsions of substituents in the aromatic ring and C_3-side chain have to be minimized. The regioselectivity in the oxidative coupling reactions of phenols may be due to different configurations of intermediate π-complexes. [55–58] These π-complexes (sandwich model) and σ-complexes (quinone methide intermediates) and the structures generated from these combinations are illustrated in Figure 7. The σ-complexes – quinone methide intermediates – are formed through the π-complexes resulting in the transition states. The quinone methides may be in equilibrium with the π-complexes or with each other, for example, through transition states such as the structure X in Figure 8 (Paper I). The nucleophilic attack of R-OH is in principle also a reversible reaction, and it is competing with the intramolecular nucleophilic attack. [59–62]

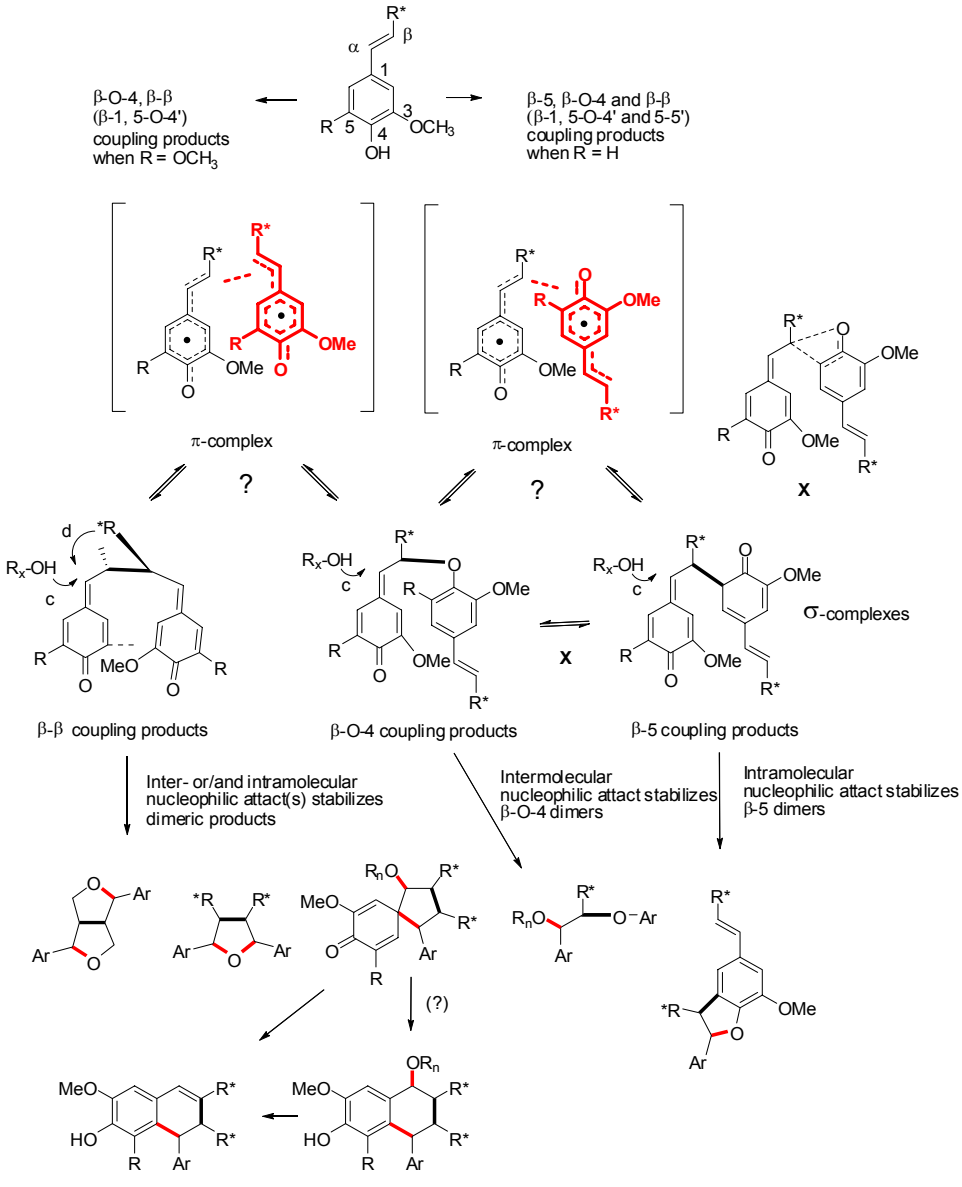


Figure 8. Schematic diagram of the possible routes and mechanisms to different structures.

2.2.2 Stabilisation of the α-carbon in quinone methides by the addition of nucleophiles and the formation of stable structures

Stabilisation of the α-carbon in quinone methides (QM) by the addition of nucleophiles is dependent on the nature and availability of nucleophiles in the reaction media [63, 64], on the structure of the QMs [63, 65, 66], on the solvent system (solvolysis, H-bonding with substrates, and bulk effects) [67, 68], and on the type of catalysis (acid or base catalyzed, pH-dependence, solvent catalysis, etc.). [62, 69] The reactivity is mainly due to the electrophilic nature of QM, which is remarkable in comparison to that of other neutral electrophiles. QMs are good Michael acceptors, and nucleophiles are readily added under mild conditions to the QM exocyclic methylene group to form benzylic adducts. [59–61, 70, 71] The formation and subsequent reactions of QMs have been shown to be highly responsive to the presence of electron-withdrawing and -donating groups in the aromatic ring: electron-donating groups greatly facilitate initial QM generation and electron-rich QMs react much more slowly but more selectively with nucleophiles than do the electron-poor QMs. [66] The reaction of nucleophiles to a quinone methide can be an intramolecular attack of a substituent of a dimeric intermediate, such as in the formation of resinols or β-5 dimers, or an attack by other nucleophiles existing in the reaction media. The reactivities of quinone methides have been observed to be influenced by both intermolecular and intramolecular interactions affecting the relative contributions of the resonance forms shown in Figure 9. The solvent effects and the effects of the structure of quinone methide to its reactivity and reaction rate (also with water) have been investigated by Bolton et al. [63] The influence of quinone methide reactivity on the alkylation of thiol and amino groups has also been studied. [72] The general observation was that quinone methides can be expected to combine rapidly with cellular nucleophiles. The reactivity of the quinone methide and, for example, the sensitivity of the QMs solvolysis reaction to the polarity of a solvent system as well as the transition state in the nucleophilic addition reaction were observed to be dependent on the structure and substituents in the aromatic ring and at the exocyclic methylene group. The transition state in the nucleophilic addition is either a highly polar transition state or an uncharged cyclohexadienone structure (see Figure 9). Modica et al. [64] have observed that at lower pH – especially at pH 2, 5, or 6, or even at pH 7 – water is a rather good nucleophile for attacking a quinone methide as compared to amino or even to sulphur nucleophiles in water solutions. Besides pH, the

ratio of addition products was dependent also on other reaction conditions such as the structure of competing nucleophiles and their nucleophilicity. Modica et al. [64] didn't observe any addition products by carboxylic acid groups for tested amino acids. The formation of β-O-4 lignin models was also studied by computational methods such as the density functional theory (DFT) method. The results showed also that the conversion of a β-O-4 linked quinone methide into a quaiacylglycerol-β-coniferyl ether dilignol is catalyzed by acid. [53]

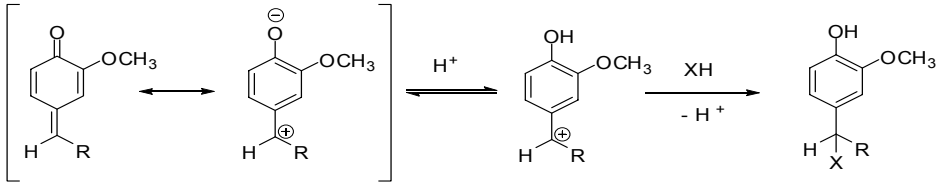


Figure 9. Structures of quinone methides and the addition of a nucleophile XH in an acid catalyzed reaction. R = -CH_3, -CH_2CH_3, -COOEt, -CHF_x, or alkyl chain with other substituents such as -$CH(OAr)CH_2OH$ in β-O-4 intermediate of coniferyl alcohol.

Nucleophile selectivities for reactions of 4-$MeOC_6H_4CR^1(R^2)Y$ with alkyl alcohols and water have been studied. [65] It was found that methanol was the most reactive substance with the carbocation 4-$MeOC_6H_4CH(CH_3)^+$ and that its nucleophilic selectivity was twenty times the selectivity of water. A similar observation was made when the reactivity of *o*-hydroxybenzyl alcohol (oHBA) with solvents like methanol, ethanol, and benzyl alcohol was studied. Methanol reacted with oHBA forming 94-% methoxy ether. [68]

2.3 Stereoselectivity in the oxidative coupling reactions of phenols

The coupling of two phenoxy radicals leads to new asymmetric stereocenters. The reaction can lead to pure enantiomers or mixtures if stereocontrol exists due to a catalyst and/or matrix and/or chiral auxiliarities in the starting compound. Enantioselective (bio)synthesis will be discussed further in Section 4.5. So-called *erythro* and *threo* isomers can be formed in the β-O-4 type coupling. The *erythro*/*threo* ratio of β-O-4-structures is an important structural characteristic of

lignin. [73] The stereochemistry in the forming of these isomers is nowadays believed to be kinetically controlled. [24] The *erythro*/*threo* ratio is observed to be approx. 1:1 in softwood species, but the *erythro* form is predominant in hardwood species. [73] A quinone methide intermediate of syringyl-type was observed to convert more frequently to an *erythro*-form than a quaiacyl-type. The highest *erythro/threo* ratio in lignins was observed to be more than 3. [74] The formation mechanism is illustrated in Figure 10 and compared to the situation in the β-5 type coupling. Many experiments *in vitro* have shown rather variable results and the clear fundamental conclusions of the correlations between the *e/t*-ratio and the reacting species, i.e. quinone methides and nucleophiles, the effect of the structure of monolignols, and/or the meaning of reaction conditions are very difficult to make. The reason is that 1) there are too few reliable experiments published so far, 2) the reaction conditions vary very much between the studies and the comparison is diffucult, and 3) real systematic studies which would take into account the most important reaction parameters in the same study are missing. Some examples are presented in Table 2.

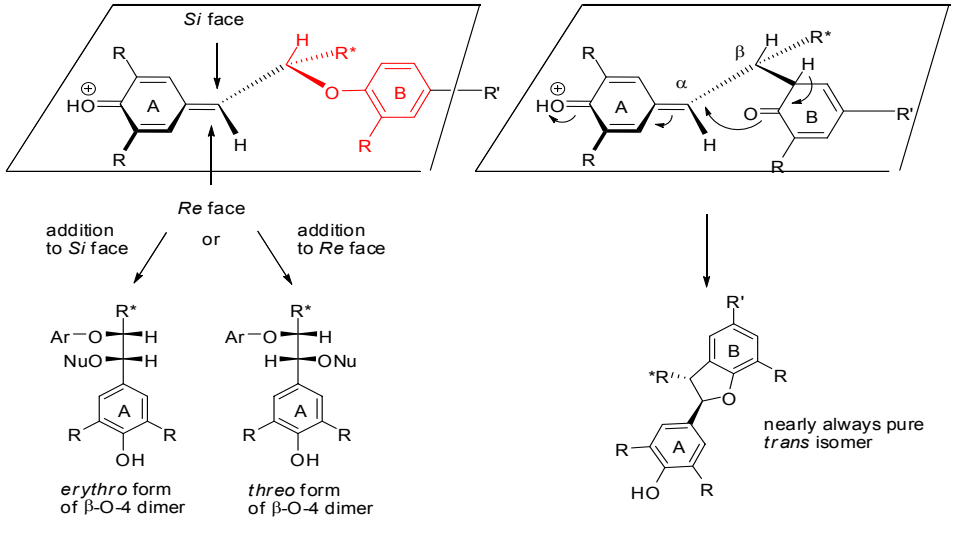


Figure 10. Schematic diagram showing the acid catalysed addition of a nucleophile to a quinone methide intermediate and the formation of erythro and threo isomers of β-O-4 dimers and the stereostructure of the C_α-C_β trans configuration in β-5 dimers (Paper I). [75]

Table 2. Erythro/threo ratios of some selected β-O-4 dimerizations. IEG = isoeugenol, CAL = coniferyl alcohol.

Monolignol(s)	Reaction conditions	substituent at C_α	*erythro* / *threo*	Ref.
IEG	Ag_2O, dry benzene + water + 1 M HCl	-OH	1	[76]
IEG	H_2O_2/HRP, 38-% aq. acetone	- OH	3,0	[77]
IEG	Ag_2O, dry CH_2Cl_2 + MeOH with pTsOH	-OMe	1	Paper I
IEG	Laccase	-OH	0,2	[78]
CAL	H_2O_2/HRP, 10-% aq. methanol	-OMe	1	Paper I
CAL	Ag_2O, dry acetone + water with 1 M HCl	-OH	1	[79]
CAL	Ag_2O, 1:2 acetone-water pH 2.5 (pH 3.1)	-OH	0,7	[79]
CAL +apocynol	$Mn(OAc)_2$ in acetic acid	-OAc	1,9	[80]
5-MeO-IEG	Ag_2O, dry benzene + water with 1 M HCl	-OH	1,4	[76]
5-MeO-IEG	Ag_2O, dry benzene + AcOH	-OAc	3,2	[76]
5-MeO-IEG	Ag_2O, dry benzene + MeOH + pTsOH	- OMe	3.0	[76]
5-MeO-IEG	Ag_2O, dry benzene + PhOH + Et_3N	- OPh	10	[76]
5-MeO-IEG	$FeCl_3$, 38-% aq. acetone	-OH	2,3	[81]

2.4 Role of peroxidases

Peroxidases are usually heme-containing glycoproteins that can catalyze various oxidative reactions including the oxidative coupling reaction of phenols. [82, 83] The oxidation potentials and the power of catalysts are different and are dependent on the catalyst's (enzyme) own structural features. [83, 84] For example, when three peroxidases such as lactoperoxidase (LPO), horseradish peroxidase (HRP), and chloroperoxidase (CPO) were compared in the oxidation of phenolic sulfides, remarkable differences were observed. With CPO the major product was a sulfoxide, but also HRP produced sulfoxides. Dimeric phenols were yielded as main products with HRP and LPO. [85] The prosthetic group of peroxidases is commonly a porhyrin-like organic molecule containing a metal atom in the centre. Usually iron but also other metals such as manganese have been observed. [86] The structures of several peroxidases have been determined,

for example, lignin peroxidase (LiP) [87], horseradish peroxidase (HRP) [88], manganese peroxidase (MnP) [86, 89] from plants, and lactoperoxidase (LPO) from mammalians. [90] HRP and its isoenzymes and other similar peroxidases in plants are believed to be the most important enzymes in the dehydrogenative polymerization of monolignols to lignin. [24] Peroxidases use hydrogen peroxide (H_2O_2) as an oxidant in the dehydrogenative dimerization and polymerization (see a review about HRP by Veitch 2004). [91]

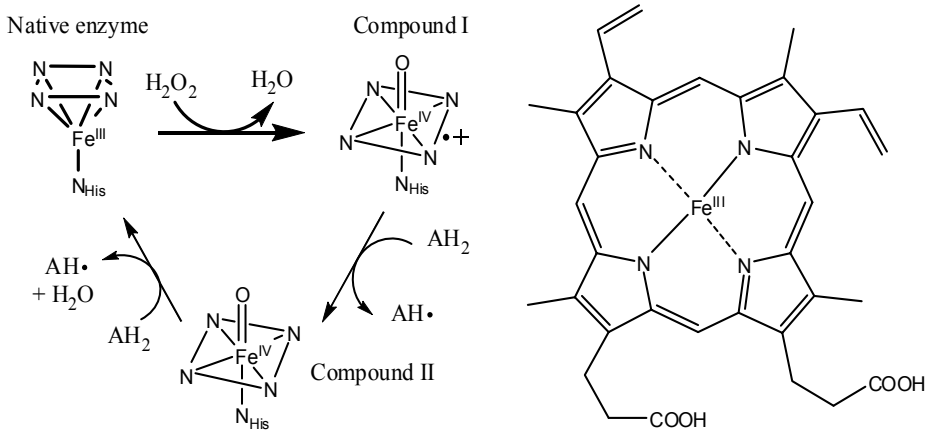


Figure 11. Catalytic cycle of horseradish peroxidase (HRP) and the structure of heme – ferriprotophorphyrine IX which is the prosthetic group of HRP and many other peroxidases. [91, 92]

Laccase has also been used to catalyze the oxidative coupling of phenols. Laccase (1.10.3.1) is a special polyphenol oxidase involved in the lignification of plant tissue and in the phytopathogenicity of several fungi. It has wide substrate specificity for phenolic compounds. It uses oxygen as an oxidant and copper(II) is involved in the reaction mechanism. [93]

The stability and activity of an enzyme is often dependent on the concentration of an oxidant which can inhibit the enzyme at a too high a concentration. [94, 95] The activity and stability of an enzyme is dependent, for instance, on the concentrations of reacting substrates, solvent type and system, ionic strength, and pH. [96] Enzymes have usually a pH optimum. [97] The tolerances of LiP and MnP (from a fungus *Bjerkandera* sp. strain BOS55) to water miscible solvents was rather limited, but MnP was more stable in acetone and ethanol. [98] MnP was also found to be more tolerant than LiP in organic solvents. [99]

The activity of MnP was studied in aqueous organic media at pH 4.5 with several water miscible solvents by using guaiacol and 2,6-dimethoxyphenol as substrates. The activity was still rather good, for example, in 70-% acetone, diethylene glycol dimethyl ether and 2-propanol, but was greatly dependent on the substrate, also. No activity was observed in 70-% methanol. [99] Meanwhile, LiP from *Phanerochaete* chrysosporium was very active in many organic solvent-water mixtures. [100] HRP has been found to be remarkably active even at high concentrations of organic solvent [101] and the effect of organic solvent on its structure and function was studied. [43] According to the kinetic studies, the apparent K_m values (enzyme-substrate interactions) in dioxane/water mixtures increased as the substrate hydrophobicity increased, whereas in aqueous buffer, the apparent K_m values remained relatively constant. Values of V_{max}/K_m were reduced because of a stronger binding of substrates to HRP (V_{max}, catalytic turnover). [43, 101]

Most peroxidases have a low substrate specificity and they can oxidize many kinds of substrates. The control of the lignification process by peroxidases may still appear in many ways. They may be more active to catalyze the oxidation of one monolignol than another, leading to accumulation of one monolignol into a growing lignin polymer. For example, some peroxidases such as syringyl peroxidase [102, 103] or cell-wall-associated oxidases [104] are found to have higher substrate specificities. Peroxidases in the close proximity of their active centre may direct the coupling of phenoxy radicals in a regioselective or in another way, leading more likely to a specific combination of two phenoxy radicals in the coupling stage and also during the post-coupling reactions to stabilized end-products. [103] The possible directing functionality and mechanism may be based on the structural diversity of the enzyme (protein part which controls in many ways the oxidation potential of the enzyme and together with the structure of heme having different kinds of metals) leading to different kinds of water activity and apparent pH near the active centre of the peroxidase enzymes. [92, 105, 106]

Peroxidases have not been observed, so far, to catalyze the oxidative coupling of phenols in an enantioselective way without the help of so-called dirigent proteins, and not at all in the case of the lignification process. [24]

So, we always have to keep in mind the fact that an enzyme (catalyst) itself may have remarkable and unexpected effects on the regio- and stereoselectivity in the

oxidative coupling of phenols, and that the effects of organic solvents on the catalytic activity and substrate specificity of enzymatic catalysis are important.

2.5 Chirality of lignans

Lignans are usually obtained from plants as their pure enantiomers, in other words, they are optically active. [17, 23] Several enzymes are responsible for their formation by catalyzing many kinds of reactions in a stereoselective manner: for example, the enantiospecific conversion of (+)-larreatricin (dehydrodiisoeugenol -like lignan with a furan ring) into (+)-3-hydroxylarreatricin by polyphenol oxidase in *Larrea tridentate* [107] or, for example, the stereoselective coupling of coniferyl alcohol to an enantiopure pinoresinol by a so-called dirigent protein found and studied by Davin and Lewis (see Figure 12). [17, 108]

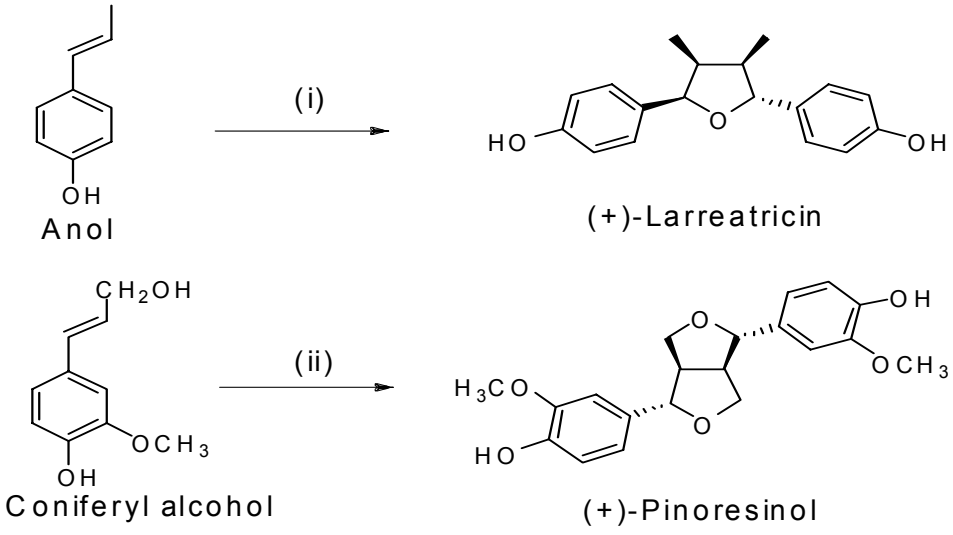


Figure 12. (i) Proposed biosynthetic pathway to (+)-larreatricin (L. tridentata) lignan [107], and (ii) the stereoselective coupling of coniferyl alcohol to an enantiopure (+)-pinoresinol by a dirigent protein in F. intermedia. [108]

Theoretically there are three basic possible reasons for this optical activity:

1) Enantioselective control of chiral catalysts such as enzymes. For example, the biosynthesis of optically pure lignans with the help of dirigent proteins. [109]
2) A chiral matrix or environment controlling stereoselectivity.
3) Chiral auxiliary substituents in the starting molecule inducing stereoselectivity. This route to chiral lignans or dilignols have been proved to be a valuable choice (Paper IV). [110]

Some peroxidases or related oxidoreductases are capable of catalyzing other kinds of oxidative reactions in an enantioselective way, for example, epoxidations of double bonds by chloroperoxidase or vanadium peroxidase [83], or sulfoxidation by HRP. [111] So, in principle, enantiocontrol may be possible also in the oxidative coupling reaction of phenols by peroxidases alone without dirigent proteins. The theory as to the existence of enantiocontrol in the oxidative coupling of phenols in the lignification process proposed by Davin and Lewis [17] is not yet accepted, or there is not been enough proof that it is involved in lignification in plants. [24, 25]

Because of the biological activity of lignans and their many potential pharmaceutical properties, syntheses of podophyllotoxin and related compounds as well as other lignans have been studied intensively. [112] For example, podophyllotoxin which is an aryltetralin lignan and its derivatives, are nowadays important drugs against cancer. [113] The antitumour activity of several products of the dihydrobenzofuran-type lignans have also been demonstrated by Pieters et al. [114] Because of the need of enantiopure lignans, several synthetic strategies for stereoselective and asymmetric synthesis of lignans have been performed to achieve aryltetralins such as podophyllotoxin [115, 116], dibenzylbutyrolactones such as matairesinol, arctigenin, or enterolactone [117, 118], dibenzylbutanediols [119], or furofuran lignans such as pinoresinol. [120] Currently, the biomimetic oxidative coupling of monolignols involving a chiral auxiliary substituent in the starting compound has also been used to produce enantiopure lignans such as dihydrobenzofurans (Paper IV) and aryltetralins. [121]

Many of these lignans used for the production of pharmaceuticals such as podophyllotoxin are extracted and purified from plants [32] and modified then chemically to the end-products. [113] Biotechnical routes to the production of enantiopure lignans may also be potential with the use of cell cultures, etc. [122, 123]

Kinetic resolution by lipase-catalyzed acetylation has been used successfully to produce enantiopure dihydrobenzofuran-type neolignans. [124] Chiral resolution is related to this technique because pure enantiomers are separated from their racemic mixtures by using, for instance, chiral chromatography (Paper VI). [125] These dihydrobenzofuran (phenyl coumaran) lignans have also been purified from their diastereomeric (-)-camphanoyl derivatives to pure enantiomers by using normal liquid chromatography. [126]

2.6 Role of cinnamic acids as crosslinking compounds in plant hemicelluloses

4-Hydroxycinnamic acids such as ferulic acid and sinapic acid play a remarkable role in the texture of cell walls where they are bound to hemicelluloses and partly dehydrodimerized forming crosslinkages between hemicellulose chains. [127, 128] They also possibly functions as linkages between lignin and cell wall polysaccharides and cellulose. [129–131] Dehydrodiferulates are likely to be the most important arabinoxylan cross-links in cereals and grasses in general. β-β (8-8’), β-5 (8-5’), β-O-4 (8-O-4’), 4-O-5, and 5-5’ dehydrodiferulates and their esters with carbohydrates have been isolated and characterized in a whole range of plant materials. [132, 133] Their oligomers have also been identified as existing in plants. [134, 135] Sinapate dehydrodimers and sinapate-ferulate heterodimers are also obtained in many plants. [136] The dimeric structures of dehydrodiferulic acid are presented in Figure 13. [129]

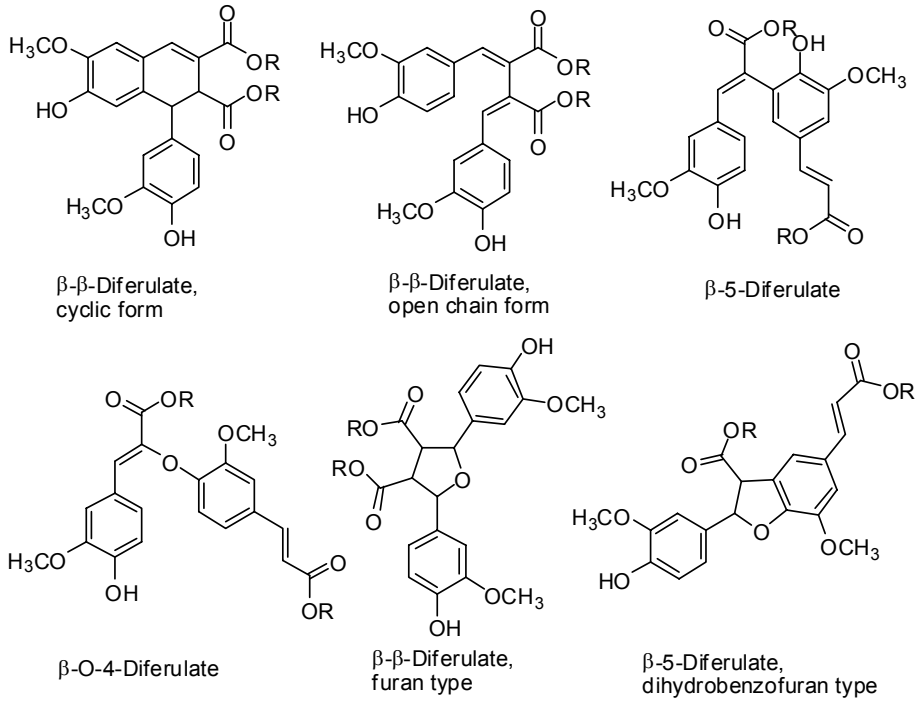


Figure 13. Main dimeric structures of dehydrodiferulic acids in plants. [129]

2.7 Formation and structure of lignin

Lignins are amorphous phenolic polymers and the second most abundant organic material in nature after cellulose. Lignins are an essential component of the woody stems of arborescent gymnosperms and angiosperms in which their amounts are in the range from 15 to 36-%. Lignins are found as integral cell wall constituents in all vascular plants including the herbaceous varieties. The lignin in the cell wall is intimately mixed with the carbohydrate components. Lignin is an essential component of higher plants giving them rigidity, water-impermeability, and resistance against microbial decay. The basic character of lignin is the lack of a regular and ordered structure. [3, 24, 25] Lignins are not optically active in contrast to lignans. [137] Usually lignin has to be extracted and isolated from plant material before structural analyses and characterization techniques by using chemical and physical methods which can change and partly destroy the natural structure of lignin. These facts make the characterization of lignin

structure rather difficult. [138, 139] Some examples of the typical softwood and hardwood lignins and of the lignins of some wood species and other plants are presented in Table 3.

*Table 3. Approximate composition (%) of some important classes of lignin with different kinds of phenylpropane units in lignin and in some selected wood and plant species. (**G**) guaiacyl, (**S**) syringyl and (**C**) p-coumaryl.*

Lignin type	**G**-type	**S**-type	**C**-type	Ref.
Softwood lignin	95	1	4	[139]
Hardwood lignin	49	49	2	[139]
Grass lignin	70	25	5	[139]
Compression wood (CW)	70	0	30	[139]
Birch (*Betula verrucosa*)	24	76	nd	[140]
Poplar (*Populus euramericana*)	49	51	nd	[140]
Spruce *(Picea abies)*	98	tr	2	[140]
Pine *(Pinus pinaster)* (CW)	82	tr	18	[140]
Rhubarb	4	96	nd	[134]
Pear	45	55	1	[134]

Thioacidolysis is a widely used method for the characterization of structural units of lignin and their amounts in lignin. [141] The use of acetyl bromide (AcBr) is also familiar to lignin chemistry because lignocellulosic material can be dissolved in acetic acid by this method. [142] The so-called derivatization followed by reductive cleavage (DFRC) method has been derived further from the AcBr method by combining the reductive step and use of Zn, and is currently also popular. [143, 144]

Many kinds of NMR spectroscopic techniques have been recently developed and have became the most powerful techniques for structural analysis of lignin. [145–147] Combining ^{13}C-labelling of DHP and wood species (*P. Abies*) suspension cultures with the use of NMR techniques such as 3D HMQC-HOHAHA is a

very interesting and powerful technique and it can yield important information regarding the chemical processes involved in the lignification of cell walls in vascular plants. [148–150] Labeling with other nuclei such as deuterium is also used. [15]

Molecular modelling has also been used to determine the comformational characteristics and to establish the structure-property relationships of biopolymers such as cellulose and lignin. [151, 152]

In addition to the modern powerful NMR techniques, "studying lignin-biosynthetic-pathway mutants and transgenics provides insights into plant responses to perturbations of the lignification system, and enhances our understanding of normal lignification". [153]

Perhaps the most important method combined with NMR techniques has been the studies with model lignins by generating so-called dehydrogenative polymers, DHPs. [154, 155] The advantage of synthetic DHPs is that they are free of carbohydrates and other wood components which can complicate interpretation of experimental results. [156] The syntheses of dimeric, trimeric, and oligomeric model compounds have given a lot of information about the basic factors in the oxidative coupling of phenols and have provided useful data in support of the characterization and idenfication methods (NMR, MS, etc.). [59–61, 70, 79, 80, 134, 157]

The currently widely accepted theory is that lignin polymer is formed by combinatorial-like phenolic coupling reactions, via radicals generated by peroxidase-H_2O_2. The reactions have been reviewed and discussed, for example, by Ralph et al. [24, 25] The cross-coupling reaction is important when the growing lignin polymer is reacting with monolignols or dilignols in the so-called dehydrogenative polymerization process (DHPs) and in the lignification process in nature. The theory on the combinatorial-like formation and polymerization process of lignin is based on the idea that lignification is a very flexible process producing complex racemic aromatic heteropolymers – lignin. According to this theory only the simple chemical coupling properties during the lignification process constrain the synthesis to limited structural diversity, and at the moment all evidence seems to point to the polymerization process itself being independent of protein/enzyme control. [25]

Another theory called “regiochemical control of monolignol radical coupling: a new paradigm for lignin and lignan biosynthesis” has been proposed. [17, 158] This theory is not discussed in more detail in this thesis because it is not approved and it has not been proven to explain the lignification process in any new way or with any adequate evidence. The debate has been going on very intensively elsewhere. [24, 25] A so-called replication theory is proposed by Sarkanen et al. [159] as one explanation in which the lignin macromolecules, without participating covalently in the process, are assumed to be able to act as template species in promoting the oxidative coupling of monolignols to form high molecular weight dehydropolymerisate components.

The feeding rate of monolignols into the dehydrogenative polymerization process is highly dependent on the hydrolytic enzymes which are one part of the control system of lignification. [160] The feeding rate of monolignols [80] and the ratio of monolignol / peroxidase [44] were shown to have a significant effect on the dehydrogenative polymerization. In the traditional Zutropf (ZT) method the monolignols are fed rapidly into a reaction mixture at the same time, whereas in the Zulauf method monolignols (and/or other reagents like H_2O_2) are added very slowly. The Zutropf method favours the formation of dimeric products whereas the Zulauf method yields more lignin-like DHPs, for instance, with higher content of β-O-4 structures.

In addition to the three predominant monolignols, *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol, it has been observed that many other monomeric phenolic compounds are involved in dehydrogenative polymerization as building blocks of lignins (see review Ralph et al. [24]).

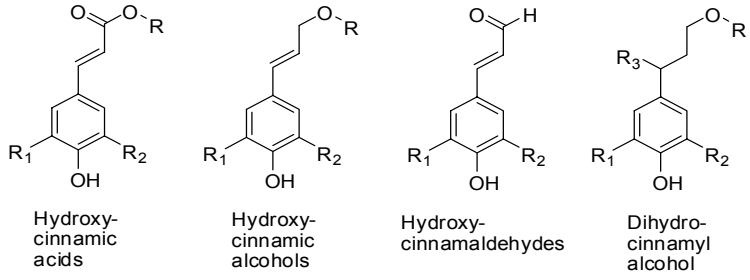


Figure 14. Some other possible monomeric phenols as building blocks of lignin where R can be carbohydrate, acetate, etc. R_1 and/or R_2 are -H or -OCH_3, R_3 = -H or -OH. [24]

Hydroxycinnamyl aldehydes have been observed to exist in lignins. [161] Hydroxycinnamyl acetates such as sinapyl acetate have also been determined in lignin. [162] Coumaroylated lignin units – esters of *p*-coumaric acid with Cγ-hydroxyl groups have been detected in bamboo and maize. [163] These few examples will already illustrate the enormous diversity of lignins in nature.

The structural feature of lignin polymers is that coupling is possible only in a certain way between monolignol and the growing lignin polymer. The main possible structural units present in lignins are illustrated in Figure 15. One of the main linkages in the lignins are β-O-4 (**A**) and β-5 structures (**B**). [24] A 5-5' coupling unit (**C** and **F**) exists usually as a dibenzodioxocin (**C**) which is a trimeric construction unit in lignins. [164] The β-β coupling units are obtained in lignins mainly as resinol (**D**) structures like pinoresinol and syringaresinol which can be principally formed through two different routes. [165, 166] The 5-5'- (**C**) and 4-O-5'-structures (**E**) are important branching points in lignin. [24] Some reduced lignin sub-structures cannot be directly explained by radical coupling reactions and nucleophilic attack exist also in lignin. The quinone methide intermediates are reduced in these structures yielding, for example, 1-(quaiacyl)- and (syringyl)propanol and secoisolariciresinols. [167] Benzodioxane (**G**) structures are also obtained in some plants such as poplar where 5-hydroxyconiferyl alcohol (caffeyl alcohol) is incorporated into lignin. [168] These main structural units are presented in Figure 15.

The relative redox potentials of monolignols have been assumed to be one possible controlling factor in the dehydrogenative polymerization, i.e. in DHPs synthesis and lignification. [45] Neudörffer et al. [169] have measured the oxidation potentials of several 4-hydroxycinnamic ethyl ester derivatives and related dehydrodimers. Oxidation potentials decreased in the order ethyl sinapate (– 0.13 V) > ethyl ferulate (0 V) > ethyl coumarate (+ 0.2 V). Hapiot et al. [170] have measured one-electron redox potentials of coniferyl alcohol and analogues. They observed that the conjugation of the phenyl ring with the double bond makes the oxidation easier, and that the addition of a methoxy group *ortho* to the hydroxyl function makes the substrate more easily oxidized and still more if there are two methoxy groups. They got a so-called formal potential value of 0.11 V for coniferyl alcohol (lifetime 5 μs) and 0.07 for isoeugenol (lifetime 20 μs). The measurements were carried out in basic acetonitrile. They also came to the conclusion that because of the rather narrow potential range of monolignol redox

potentials, the differences of reactivity observed for monolignols in lignin polymerization must result from kinetic effects of the reactions following the first electron transfer. The aqueous oxidation potentials were measured to be 0.64 eV for coniferyl alcohol and 0.50 eV for sinapyl alcohol by Wei et al. [171] The substituent effect on the O-H bond dissocation enthalpies of phenols is studied by EPR radical equilibrium techniques. [172] Russell et al. [173] used also EPR methodology to investigate the effects of substrate structure on peroxidase-catalyzed phenylpropanoid oxidation and demonstrated that the structure of the monomer or dimer determines the final composition of lignin.

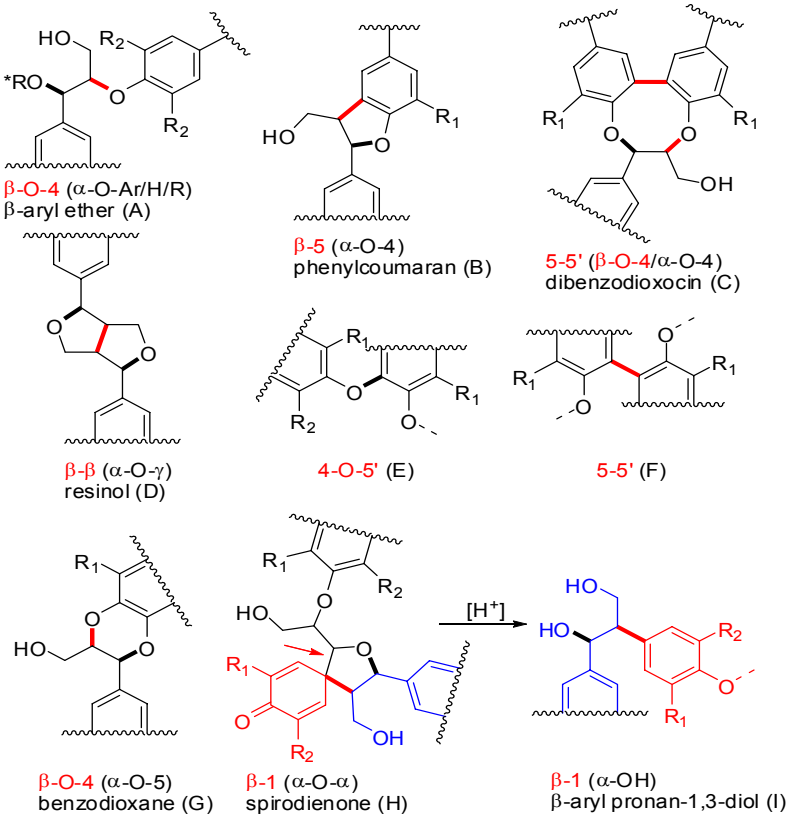


*Figure 15. The main structural units in lignins. The primary C-C or C-O bond formed in the oxidative coupling of two phenoxy radicals is **bolded** and **red**. The post-coupling bonds are **bolded** and **black**. The red arrow shows the first breaking bond when the "traditional" β-1 aryl propan-1,3-diol structure is formed from the β-1 spirodienone structures. R_1 is -OCH_3 and R_2 are -H in guaiacyl units and R_1 and R_2 are -OCH_3 syringyl units.*

Okusa et al. [174] compared laccase and peroxidase on the dehydrogenative polymerization of coniferyl alcohol and observed that the polymerization process was strongly dependent on the enzyme used. For example, laccase from *Rhus vernicifera* produces mainly dimeric products after 144 hrs: 21-% β-β, 26-% β-5 and 2-% β-O-4. DHP was not obtained. Laccase from *coriolus versicolor* produces after 51 hrs also mainly dimeric products: 13-% β-β, 22-% β-5 and 11-% β-O-4. DHP was obtained in 6-% yield, but when the amount of the enzyme was higher the dimeric products disappeared and the yield of DHP was 28-%. Laccase from *Pycnoporus coccineus* produces after 72 hrs only DHP in 96-% yield. The reaction using HRP (ZT method) was much faster and produced depending on the amount of oxidant, mainly dimeric products: 8–17-% β-β, 7–17-% β-5 and 4–16-% β-O-4, or DHP in 35–56-% yield when a larger amount of hydrogen peroxide and longer reaction times were used.

A good example of the effect of reaction conditions and matrix is a study of the effect of reaction media concentration on the solubility and the chemical structure of lignin model compounds (DHP). [175] The end-wise polymerization (Zutropfverfahren; ZT) method was used to prepare DHPs with or without arabinoxylan. The amount of β-O-4 type linkages and the molecular weight (MW) of DHP clearly increased in the arabinoxylan media. [176] Organic water-miscible solvents are observed to have a clear effect on the molecular weight and yields of phenolic polymers. For example, when the amount of acetone was increased to 30-%, the yield of polyquaiacol increased to 64-% (from 33-% in water) and molecular weight increased slightly from 1190 to 1260, but the MW was even higher in 50-% aq. acetone, 1690 g/mol. [177]

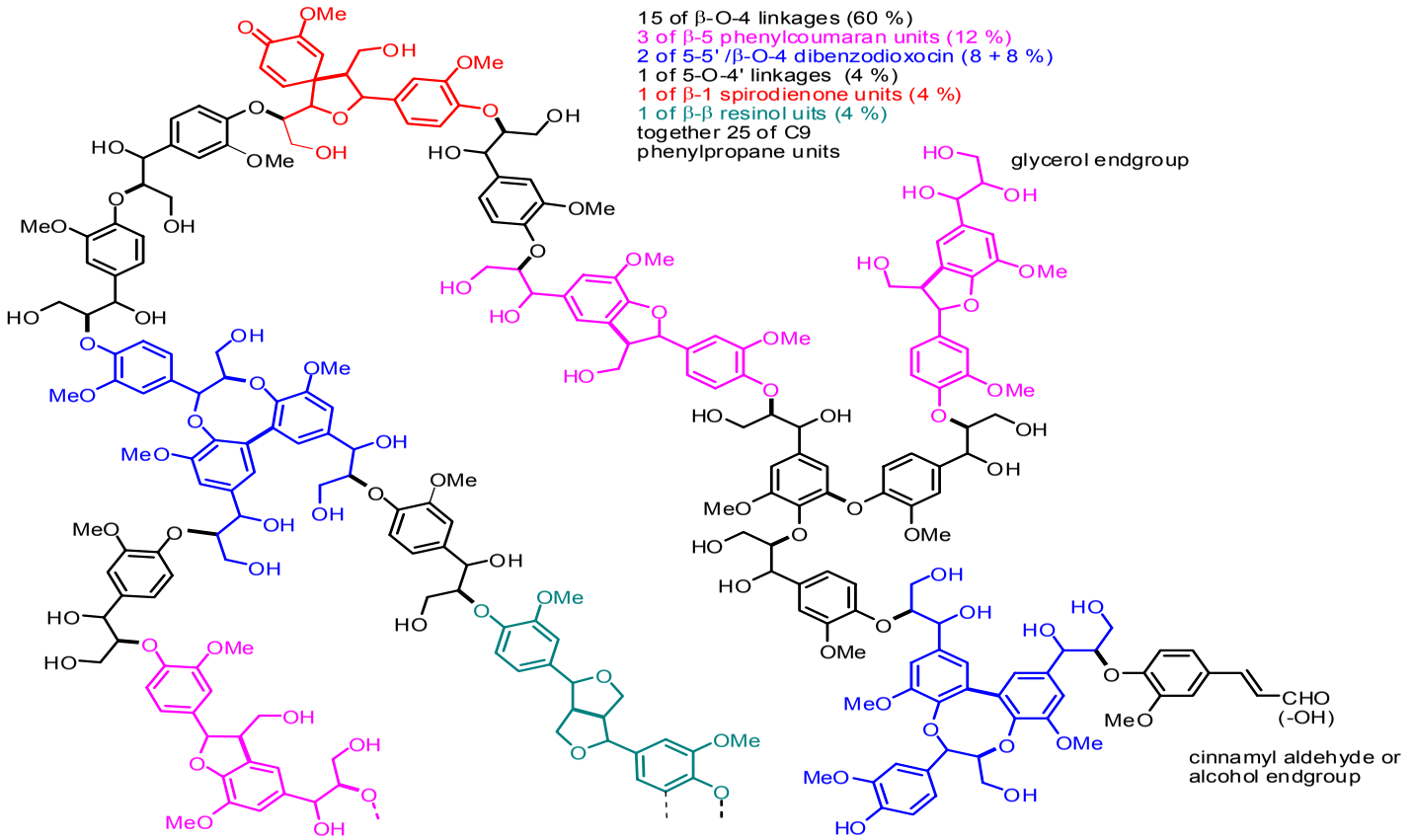


Figure 16. The model of the structure of spruce lignin with 25 phenylpropane units. Bolded bonds are from primary phenoxyradical coupling. [25]

3. Aim of the present study

The aim of this study:

- to determine the effect of the structure of monolignol, i.e., the substitution pattern in the aromatic ring and the structure of the propenyl chain, on the regioselectivity in the oxidative coupling reaction

- to determine the effect of reaction conditions, i.e., solvent and pH, on the regioselectivity in the oxidative coupling reaction

- to determine the effect of catalysts. Four different peroxidases were tested as well as some inorganic oxidation systems.

- to determine the effect of chiral auxiliarities on stereoselectivity in the oxidative coupling reaction.

- to synthesize some enterolignans and lignans in preparative scale and to resolve the pure enantiomers by using preparative liquid and/or cryogenic chiral chromatography.

4. Results and discussion

4.1 Effects of the structure of monolignols and reaction conditions on regioselectivity in the oxidative coupling reaction of phenols (Papers I, II and III)

Four different kinds of monolignols were studied in the dehydrodimerization of two similar monolignols (homodimerization): isoeugenol IEG (**1**), methyl ferulate MeFA (**2**), coniferyl alcohol CAL (**3**), and methyl sinapate MeSA (**15**). The aromatic rings of the first three monolignols have similar structures where the C-5 atom does not have a methoxy substituent and this position is free to react in the oxidative coupling yielding β-5 (or 5-5 or 5-O-4') dimers. This position in methyl sinapate (**15**) is blocked by a methoxy substituent (Figures 17 and 18).

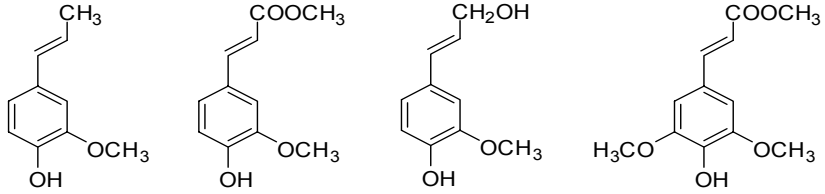


Figure 17. Four monolignols with different kinds of substitutients in the aromatic rings and in the propenyl chain were studied.

The substituents at C_γ of the propenyl chain were chosen to present the common substituents in monolignols and to give a different inductive effect and in this way to change the ratio of possible coupling products.

- The methyl substituent is an electron-releasing (+*I*) group yielding higher electron density to the β-position.
- The carboxylic acid ester group such as -$COOCH_3$ is an electron-attracting (-*I*) group thus increasing the acidity of the β-proton. This behaviour was very clearly seen when the structure of dehydrodiferulic acid and its esters were analysed from grass and *in vitro* experiments. [178]

- The methylene alcohol group -CH_2OH is a weakly electron-attracting group. This group is involved in the three main monolignols in the biosynthetic process of lignin. Furthermore, this aliphatic primary hydroxyl group can react as a nucleophile in the intramolecular addition to a quinone methide intermediate yielding, for instance, resinols or furan-like structures.

Therefore, it was assumed that these substituents at C_γ with different (stereo)electronic effects might yield clearly different ratios of coupling products and give useful experimental information concerning the mechanism and/or reaction controlling parameters pertaining to the oxidative coupling of phenoxy radicals forming the primary C-C or C-O bond at the Cγ position. The different kinds of substituents at Cγ of the monomers were supposed to affect the coupling reaction also according to Shigematsu et al. [42] After the oxidative coupling of phenoxy radicals, the formed quinone methide structures – β-β or β-5 through C-C bond, or β-O-4 through C-O bond – together with ordinary substituents at C_γ should have a different kind of inductive effect on the addition reaction of nucleophiles to quinone methide.

The yields of β-5 and β-O-4 (α-OMe) dimers were measured as a function of pH and as a function of solvent content and type of solvent by using monolignols **1**–**3** as starting materials. Other dimeric products – except pinoresinol (**10**) – were not determined, although, for example, β-O-4 (α-OH) dimer could be formed especially at pH 3–4. The reactions were first performed in a 10 mL total volume reaction mixture. The yields were determined by HPLC and synthesized model compounds were used as external standards. Some reactions were also performed in a preparative scale (ca. 1 g of starting material) and isolated yields were measured.

Methyl sinapate (**15**) was used as a model compound when the dehydrodimerization of 3,5-dimethoxy substituted 4-hydroxycinnamics was studied.

Mainly horseradish peroxidase (HRP) was used as a catalyst with hydrogen peroxide as an oxidant. The molar ratio of monolignol / oxidant was always 1:0.5 because one H_2O_2 can generate two phenoxy radicals. Other oxidants or catalysts are mentioned later in the text (see p. 49 and 52). Other peroxidases were also studied. These results are presented and discussed in Section 4.3.

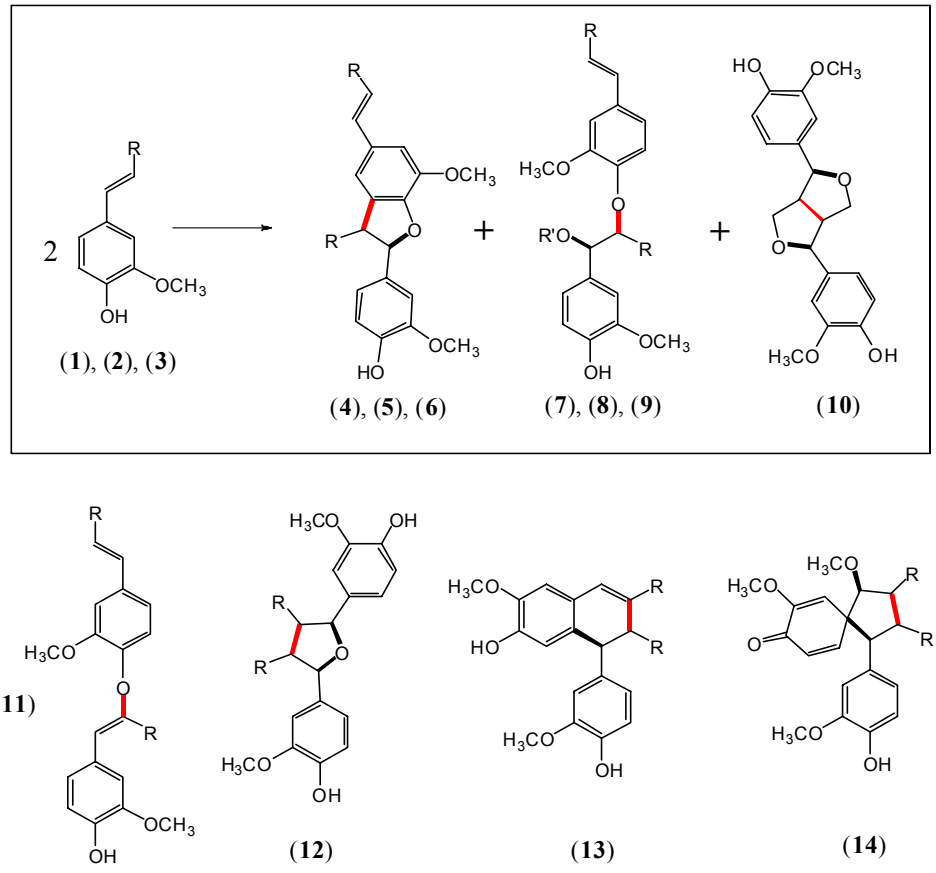


*Figure 18. Isoeugenol, R = -CH_3 (**1**) and its dimers (**4** and **7**); methyl ferulate, R = -$COOCH_3$ (**2**) and its dimers (**5** and **8**); coniferyl alcohol, R = -CH_2OH (**3**) and its dimers (**6**, **9** and **10**); R' = -CH_3 in the β-O-4 dimers **7**, **8** and **9**. The bonds formed primarily in the coupling reaction of phenoxy radicals are **bolded** and **red**. The bonds formed in the post-coupling reactions are **bolded** and **black**.*

The main products **4**–**10** identified and partly also quantified by HPLC (Paper I) are presented in Figure 18. Some other common dimeric structures (**11**–**13**) and β-O-4 dimers (**7**–**9**) with α-OH group (R' = OH) are mentioned according to the literature reviewed here. The dimeric spirodienone structure (**14**) from β-β coupling of methyl ferulate was identified tentatively from the experiments performed in water-methanol mixtures at pH 3–4 (see experimental data and discussion in Section 4.2).

4.1.1 Effect of pH and cosolvent on product distribution (Paper I)

The effect of pH was measured at pH 3, 4, 6, and 7.4 in water-organic solvent mixtures with 10-% cosolvent. Methanol and dioxane were used as cosolvents.

The yields of all of the β-5 dimers (**4**), (**5**), and (**6**) were clearly dependent on pH (see Figure 21). The highest yield of β-5 dimer with every monolignol seemed to be at pH 3–4 in water-methanol mixtures (10-% MeOH). The yields of β-O-4-α-OMe dimers were rather low. The more detailed results are presented and discussed below.

Isoeugenol (1) as starting material

The yield of the β-5 dimer (**4**) of IEG was high (64-%) in 10-% aq. methanol at pH 3 but decreased to 28-% at pH 7.4 (Paper I). A similar low yield, 19-% of the β-5 dimer (**4**), was also obtained elsewhere in 28-% aq. methanol at pH 6 according to Krawczyk et al. [179] A high yield of (**4**) has been reported by Nascimento et al. [180], even as high as 99-% β-5 dimer of IEG in 10-% aqueous methanol at pH 3. The 64-% yield of β-5 dimer (+ 5-% of β-O-4-α-OMe) – published in Paper I – was obtained under the same conditions, but Nascimento et al. [180] used a higher concentration of isoeugenol as a starting material, i.e., 20 mM of isoeugenol compared to 10 mM used in Paper I. One explanation for the difference between these results might be a rather poor solubility of the β-5 dimer (**4**) of IEG into the solvent system used with a high content of water (90%). Under these conditions the β-5 dimer starts to precipitate and does not participate in the oxidative coupling reaction resulting in a higher yield of dimeric material(s). The yield of β-O-4 dimer-α-OMe (**7**) of isoeugenol was rather low at every pH: 5-% β-O-4 dimer at pH 3, 9-% at pH 4, 5-% at pH 6, and 4-% at pH 7.4, all in 10-% aqueous methanol. The yield of β-5 dimer (**4**) was rather high at every pH, although the competition of water addition or other nucleophiles seemed to be possible, especially at higher pH values leading to different kinds of products. For example, the formation of furan-like products (13-%) have been obtained with isoeugenol as a starting material by Sarkanen and Wallis. [77] The results (Paper I) and some selected references are presented in Table 4.

The highest yield, ca. 80-% of β-5 dimer of IEG, was obtained in 10-% aq. dioxane at pH 4–6 where no methanol is present. This result indicates also that water does not compete so efficiently as a nucleophile and here the intramolecular nucleophilic attack of the phenolic hydroxyl group to the quinone methide can occur more efficiently (Paper I). The yields of 65-% β-5 and 22-% β-O-4-α-OH were obtained in 38-% aq. acetone by Sarkanen and Wallis [77] – only pure water was used without any pH adjustment. Very similar results were also published by Shiba et al. [78] when the experiments were performed in 50-% aq. acetone by using laccase as a catalyst. These observations indicate that the addition of water into the quinone methide intermediate has to be still rather efficient and also the formation of other structures is possible. The effect of solvent – type and concentration – seems to be even more important than the pH effect.

The situation was different when dry organic solvents and inorganic oxidants were used. The yields of β-O-4 dimers were usually much higher. The rather stable quinone methide intermediate of a dilignol was first generated in a dry solvent by inorganic oxidants such as Ag_2O or MnTPPX/oxidant-system. A suitable nucleophile was added after the first stage with an acid as a catalyst. For example, β-O-4-α-OMe dimer (**7**) was prepared in a 59-% yield by using Ag_2O in dry dichloromethane after adding methanol with a small amount of TsOH (Paper I). Zanarotti et al. [76] prepared the β-O-4-α-OH dimer in a 62-% yield by using Ag_2O in dry benzene after adding a water-THF-HCl mixture into the reaction mixture. Kuo et al. [181] used $FeCl_3$ in aq. acetone and obtained 53-% β-5 dimer (**4**), the amount of β-O-4 dimer or other dimers were not determined.

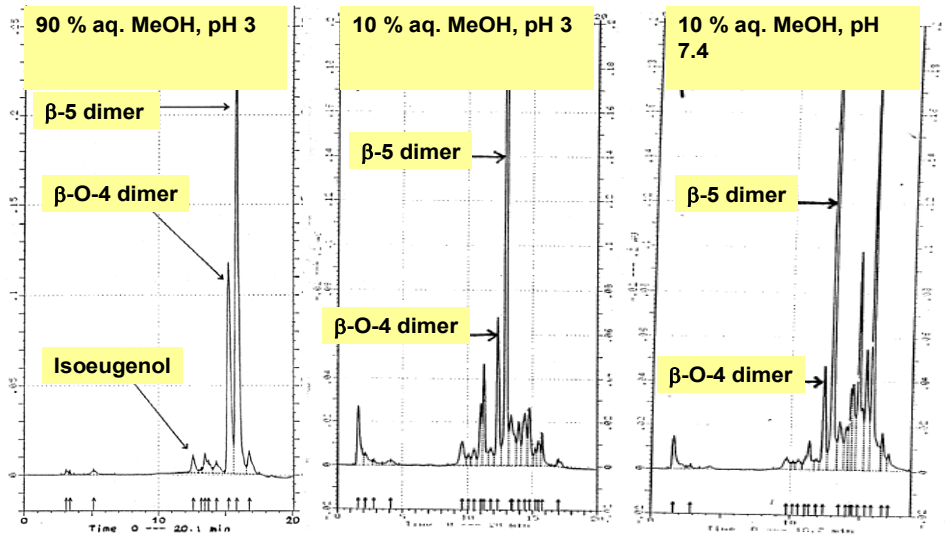


Figure 19. The effect of reaction conditions on the dimerization of isoeugenol illustrated by chromatograms (HPLC). The coupling reaction was very selective in 90-% aq. methanol at pH 3 where the two dimers formed in a yield of totally ca. 90-%. The erythro and treo isomers of β-O-4 dimer were determined to overlap in the same peak.

The amount of oligomeric and other unknown products – the peaks after β-5 dimer – increased when pH was increased up to 7.4. This can be seen clearly from the chromatograms in Figure 18. Similar behaviour was observed when apocynin (4-hydroxy-3-methoxyacetophenone) was oxidised by soybean peroxidase at different pHs by Antoniotti et al. [182] Trimeric and oligomeric products were favoured at pH 7 and much more at pH 8, and the yield of a dimeric product was highest (18-%) at pH 6. It is known also that under neutral conditions (pH 6.5) the predominant reactions are the additions of phenolic hydroxyl group to quinone methide intermediates to form benzyl non-cyclic aryl ethers both in aqueous and non-aqueouns solutions. [59–61]

All of the previous results are published in Paper I and selected examples of the results in scientific literature are presented in Table 5 (p. 56).

Methyl ferulate (2) as starting material

The yield of the β-5 dimer (**5**) of methyl ferulate (MeFA) was very similar in 10-% aq. methanol and dioxane, and it was dependent on the pH almost linearly

decreasing from ca. 50-% at pH 3 down to 10-% at pH 7.4. The β-O-4 dimer (**8**) of MeFA with similar structure compared to other β-O-4 dimers of IEG or CAL was not observed at all. This can be explained by the observation of Ralph et al. [178], i.e., when the quinone methide intermediate of β-O-4 dimer of diFA is formed, the acidic β-proton is eliminated very easily to form a conjugated structure instead of the nucleophilic attact of water or a phenol to the quinone methide α-carbon (see Figure 20). See also Table 6 (p. 57) with some results (Paper I) and selected references.

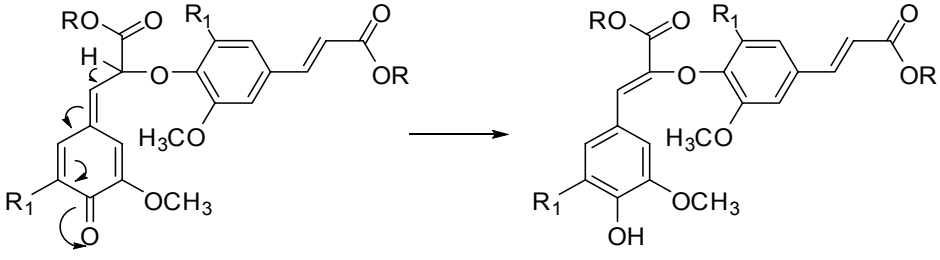


Figure 20. Elimination mechanism of the β-proton from a β-O-4 dimer of p-hydroxycinnamic acid derivatives forming a double bond. [178]

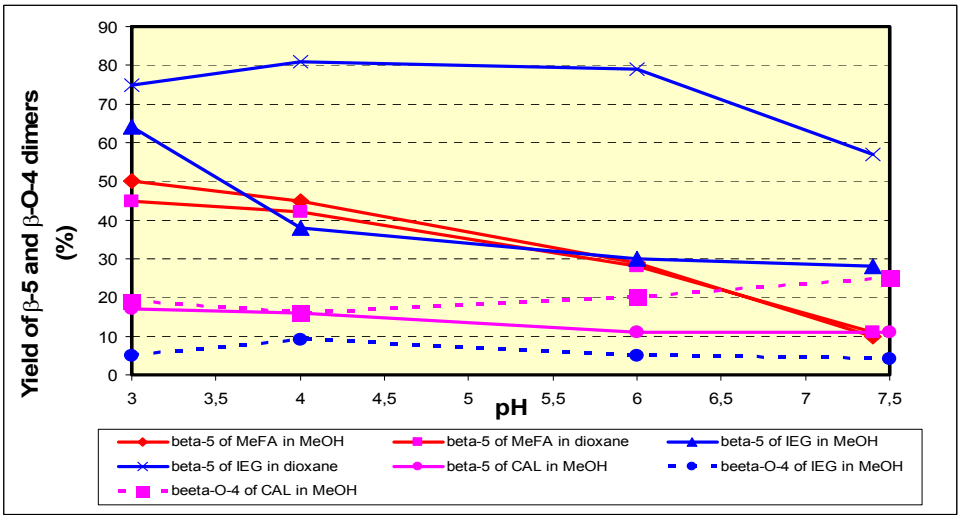


*Figure 21. The yield of β-5 and β-O-4 dimers of isoeugenol (**1**) and coniferyl alcohol (**3**), and β-5 dimer of methyl ferulate (**2**) measured as a function of pH in aqueous methanol and dioxane (10-% of cosolvent in citrate-phosphate buffer, 0.02 M). β-O-4 of (**2**) was not observed.*

Coniferyl alcohol (3) as starting material

The yield of β-5 dimer (**6**) was rather low at every pH, i.e., ca. 11–17-% in MeOH, and even lower in dioxane (< 10-%) (see Table 6, p. 58). The yields of β-O-4 (-α-OMe) dimer (**9**) of coniferyl alcohol were 19, 16, 20, and 25-% at pHs 3, 4, 6, and 7.4, respectively, and slightly increasing as a function of pH. A clear correlation between pH and the formation of β-O-4-(α-OMe) dimers was not observed. The nucleophilic addition of methanol to β-O-4 quinone methide intermediates of diCAL and also of diIEG seemed to be almost independent of pH. Competition with the other nucleophiles such as water at low pH and phenolic hydroxylic groups at neutral pH (6–7) should be always noticed. [57]

The yields of ca. 17–25-% of β-5 dimer (**6**) of coniferyl alcohol (diCAL) were very similar at pH 3–4 to the ca. 24-% yields published by Syrjänen and Brunow [80] and Quideau et al. [79] (see Table 6). Even in glacial acetic acid the yield of β-5 dimer (**6**) was 22-%. [80] The results obtained at pH 7–7.5 were also comparable to other published results, i.e., yields were ca. 10–17-%. The yield of β-O-4-α-OMe (**9**) of diCAL was at the same level as the yields of β-O-4-α-OH dimer of coniferyl alcohol reported by other researchers. [47, 79, 80, 174] The yields of β-O-4-α-OMe diCAL (**9**) were always between 16–25-% of (**9**) in aqueous 10-% methanol at pH 3–7.4 (Paper I), and 3–18-% of β-O-4-α-OH of diCAL when 20–30-% aqueous acetone at pH 3–7.4 was used as the reaction medium. [79, 80, 174] The β-β or pinoresinol structure **10** was obtained in ca. 10-% yield only in the dehydrodimerization of coniferyl alcohol and found to be nearly constant in all conditions. For example, in 90-% aqueous methanol (pH 3) its yield was 8–10-%. The yields of pinoresinol published so far have always been at the level of 5–18-% in aq. organic solvent systems with water miscible organic solvents. [54, 79, 80, 174] Therefore, in this study its yield was assumed to be ca. 10-% (Paper I) in all conditions and experiments. The total yields of measured dimers (**6** + **9** + **10**) of coniferyl alcohol were at the level 44-% (+/- 2) at every pH. The yields of the β-O-4-α-OH dimer or other possible dimeric products were not measured at different pHs. This may be one reason for the rather low yields. The dehydrodimerization of coniferyl alcohol appeared to be not so regioselective and dependent on pH as the dehydrodimerization of the two other monolignols. Another reason might be that in this case water and other nucleophiles may be much more competitive nucleophiles. [57]

Other catalysts and observations

Some inorganic catalysts and oxidants were also tested (Paper I). Often these catalysts and oxidants cause other oxidation reactions not only the oxidative coupling reaction and the yield of wanted products decreases. [183] Even so, these catalytic systems can be used as biomimetic oxidant systems because many oxidoreductases and also peroxidases can catalyze other oxidation reactions. HRP immobilized on Celite was also tested as a catalyst in the similar manner as published by Pietikäinen et al. [184] The use of a dry organic solvent as the reaction medium and the use of other catalysts/oxidants may provide useful routes to prepare model compounds which are difficult to prepare by using enzymes in aqueous media (Paper I). [76, 80]

4.1.2 Effect of organic cosolvents on the distribution of dimeric structures (Paper I)

The yields of the dimers (**4**–**6** and **7**–**9**) at pH 3 were then measured as a function of cosolvent at levels of 10, 30, 50, 70, and 90-% (v/v). Preparative scale dimerizations were performed in selected reaction conditions to ensure the results obtained at the small scale experiments.

The yields of the β-5 dimers (**4**) of isoeugenol and (**5**) of methyl ferulate first decreased to a local minimum and then began to rise as a function of the cosolvent content. HRP was rather stable even in 90-% aq. methanol because the yields still increased. HRP became inactive at higher than 70-% concentration of dioxane and no reaction was observed. The same effect has also been observed by Dordick et al. [185] The rate of the HRP-catalyzed oxidation of *p*-phenylphenol in 90-% aq. dioxane decreased to the value of 33 as compared to 308 (μmol/min mg enzyme) in 10 mM acetate buffer, pH 5. [185] Otherwise the yields were higher in dioxane than in methanol at the same concentration of the cosolvent. The reason was most likely that methanol was so effective as a nucleophile yielding a β-O-4-α-OMe dimer especially at higher methanol concentrations, and therefore decreased the yield of the β-5 dimers. When the amount of methanol was increased, the yields of β-O-4 dimers also increased. The yields of β-O-4-α-OMe dimers (**7**) and (**9**) increased almost linearly as a function of methanol content up to 21-% (**7**) and 42-% (**9**) of that dimer. Total yields of

dimers (**4** + **7**) were 88-% in 90-% aq. methanol, and the total yields of dimers (**6** + **9** + **10**) were 76-% at pH 3 in 70-% aqueous methanol assuming that the yield of pinoresinol was ca. 10-%. The very interesting observation was that the yields of β-5 dimers after a certain cosolvent content continued to increase despite the simultaneous increasing yields of β-O-4-α-OMe dimers! The yields of β-5 dimers also increased in aq. dioxane (see Figures 22 and 23). These results indicate that dehydrodimerization might be generally favoured by higher cosolvent contents as observed by Terashima and Atalla [54], too. The regioselectivity in the oxidative coupling reaction of phenols increased evidently due to higher cosolvent concentration and also at lower pH, and the dehydrodimerization may be performed with better regioselectivity yielding only two main products with some monolignols as starting materials. This is illustrated very clearly in Figure 19 (p. 50) where HPLC chromatograms are shown of the reaction mixtures from the dehydrodimerization of IEG in 90-% aq. methanol at pH 3. Total yield of these two β-5 and β-O-4 dimers of IEG was approx. 90-%. Syrjänen et al. [80] performed the dehydrodimerization of coniferyl alcohol in glacial acetic acid and they obtained a 50-% yield of β-O-4-α-OAc. Acetic acid served as a nucleophile trapping effectively the quinone methide intermediate to a β-O-4 product. However, the β-5 dimer was also obtained at a 22-% yield! The reason for the very high regioselectivity is not clear but one explanation might be the changed enzyme-substrate interactions and/or the solvent effect on the transition states leading to different reaction products. [43, 101]

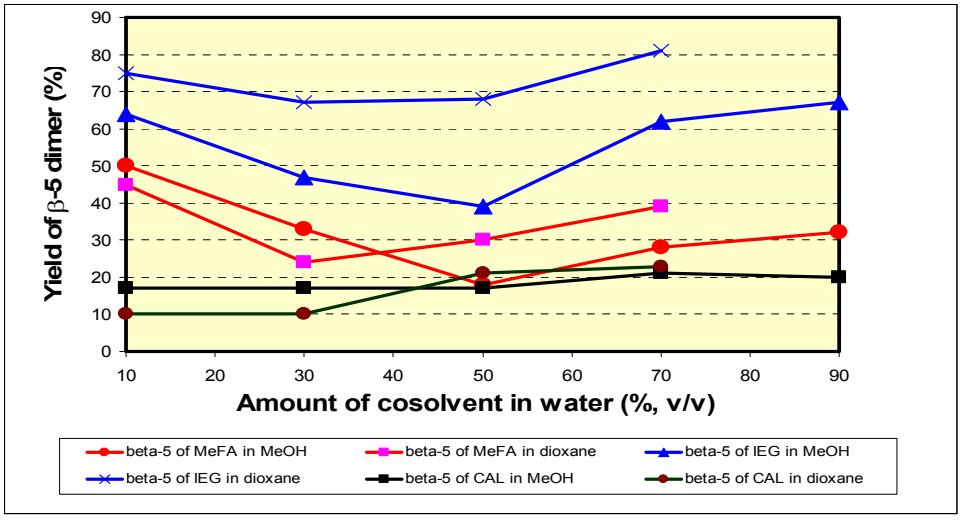


*Figure 22. Yield of β-5 dimers of isoeugenol, IEG (**1**), methyl ferulate, MeFA (**2**), and coniferyl alcohol, CAL (**3**) as a function of solvent type and content in an aqueous citrate-phosphate buffer (0.02 M, pH 3). Methanol and dioxane were used as cosolvents.*

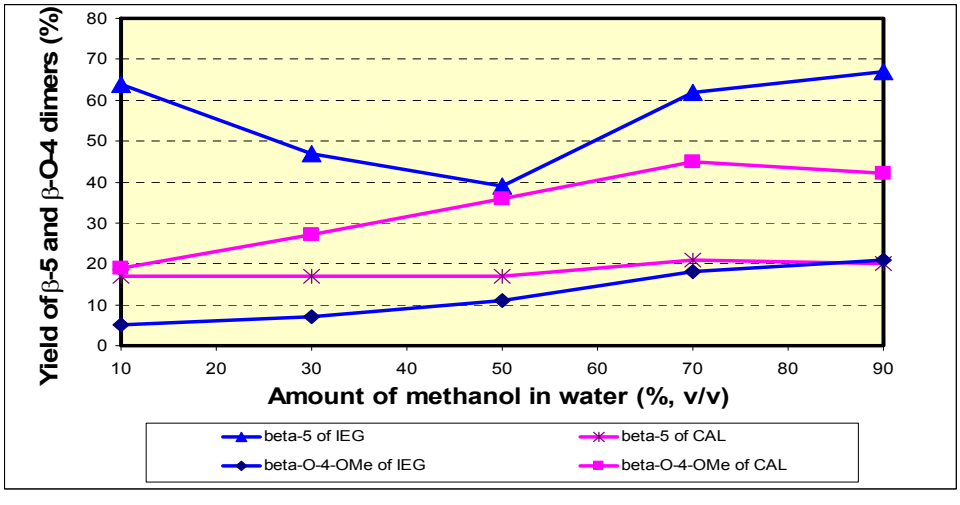


Figure 23. Effect of methanol content in water (citrate-phosphate buffer, 0.02 M, pH 3) on the formation of β-5 and β-O-4-α-OMe dimers from isoeugenol, IEG (1) and coniferyl alcohol, CAL (3).

Table 4. Dimerization of isoeugenol (1) using different kinds of oxidant systems (nm = not measured or mentioned; x = not used; xx = not formed; a) small scale screening experiments (10 mL) analyzed by HPLC; b) isolated yields from preparative scale synthesis).

Oxidant	Solvent system	Catalyst/phenol/oxidant	Isoeugenol	H_2O_2	**Yields of dimers**				Dimers	Ref.
		units / mmol / mmol	(mM)	(mM)	β-5	β-O-4 (α-OMe)	β-O-4 (α-OH/OAc)	β-β	total (%)	
H_2O_2/HRP	10-% aq. methanol, pH 3 [a]	2150 U / 4.8 / 2.4	10	5	**64**	5	nm	nm	69	Paper I
H_2O_2/HRP	70-% aq. methanol, pH 3 [a]	2150 U / 4.8 / 2.4	10	5	62	18	nm	nm	80	Paper I
H_2O_2/HRP	90-% aq. methanol, pH 3 [a]	2150 U / 4.8 / 2.4	10	5	67	21	nm	nm	88	Paper I
H_2O_2/HRP	10-% aq. methanol, pH 6 [a]	2150 U / 4.8 / 2.4	10	5	30	5	nm	nm	35	Paper I
H_2O_2/HRP	10-% aq. dioxane, pH 3 [a]	2150 U / 4.8 / 2.4	10	5	**75**	xx	nm	nm	75	see exp.
H_2O_2/HRP	70-% aq. dioxane, pH 3 [a]	2150 U / 4.8 / 2.4	10	5	**68**	xx	nm	nm	68	see exp.
Ag_2O	dry benzene (+ water + THF + HCl)	x / 5.2 / 10.4	6	x	nm.	xx	31 + 31 (e/t = 1,0)	nm	62	[76]
$PhI(OAc)_2$	dichloromethane	x / 24.4 / 7.6	140	x	35	xx	nm	nm	nm	[186]
aq. $FeCl_3$	acetone	x / 10 g / nm	nm	x	53	xx	nm	nm	nm	[181]
H_2O_2/HRP	38-% aq. acetone	2000 U / 30.1 / 16.1	94	49	**65**	xx	17+ 5 (e/t = 3,4)	13	87	[77]
H_2O_2/HRP	23-% aq. methanol, pH 6	approx. 100 U / 6 / 0.5	11	1 (?)	19	nm	nm	nm	nm	[179]
H_2O_2/HRP	10-% aq. methanol, pH 3 [b]	2500 U / 10 / 5	**20**	10	**99**	nm	nm	nm	99	[180]
H_2O_2/HRP	90-% aq. methanol, pH 3 [b]	2150 U / 7.3 / 3.6	14,5	7,3	**53**	10 + 17 (e/t = 0,6)	nm	nm	80	Paper I + see exp.
Laccase	50-% aqueous acetone	4000 U / 3.1 / O_2	155	x	**41**	nm	4 + 18 (e/t = 0,2)	nm	63	[78]

Table 5. Dimerization of methyl (or ethyl) ferulate (2) using different kinds of oxidant systems (nm = not measured or mentioned; x = not used; xx = not formed; a) small scale screening experiments (10 mL) analyzed by HPLC; b) isolated yields from preparative scale synthesis).

Oxidant	Solvent system	Catalyst/phenol/oxidant	MFA (**2**)	H_2O_2	**Yields of dimers**				oligomers	Reference
		units / mmol / mmol	(mM)	(mM)	β-5	β-O-4 (α-OMe)	β-O-4 (α-OH/OAc)	β–β	(%)	
H_2O_2/HRP	10-% aq. methanol, pH 3 [a]	2150 U / 4.8 / 2.4	10	5	50	xx	nm	nm	nm	Paper I
H_2O_2/HRP	90-% aq. methanol, pH 3 [a]	2150 U / 4.8 / 2.4	10	5	32	xx	nm	nm	nm	Paper I
H_2O_2/HRP	10-% aq. methanol, pH 6 [a]	2150 U / 4.8 / 2.4	10	5	29	xx	nm	nm	nm	Paper I
H_2O_2/HRP	10-% aq. dioxane, pH 3 [a]	2150 U / 4.8 / 2.4	10	5	45	xx	nm	nm	nm	see exp.
H_2O_2/HRP	70-% aq. dioxane, pH 3 [a]	2150 U / 4.8 / 2.4	10	5	39	xx	nm	nm	nm	see exp.
H_2O_2/HRP	90-% aq. methanol, pH 3 [b]	2150 U / 4.8 / 2.4	10	5	30	xx	nm	nm	nm	Paper I
H_2O_2/HRP	10-% aq. methanol, pH 7.4	500 (?) / 0.2 / 0.4	20	40	**7**	xx	nm	nm	nm	[188]
Ag_2O	dry benzene-acetone (2:1)	x / 0.06 / 0.04	193	x	**50**	xx	nm	nm	nm	[190]
Ag_2O	dry CH_2Cl_2	x / 0.48 / 0.24	96	x	**50**	xx	nm	nm	nm	[191]
H_2O_2/HRP	50-% aq. ethanol	? / 10.7 / 7.5	7	x	**21**	xx	nm	nm	nm	[189]
H_2O_2/HRP	acetate buffer, pH 4, 40 °C	2000 U / 9 / 6.7	5	x	50	xx	nm	nm	nm	[192]
Ag_2O	dry benzene-acetone (2:1)	x / 11.5 / 5.9	189	x	31	xx	nm	nm	nm	[125]

Table 6. Dimerization of coniferyl alcohol (3) using different kinds of oxidant systems (nm = not measured or mentioned; x = not used; xx = not formed; a) small scale screening experiments (10 mL) analyzed by HPLC; b) isolated yields from preparative scale synthesis).

Oxidant	Solvent system	Catalyst/phenol/oxidant	Conif. alc.	H_2O_2	Yields of dimers				oligomers	Ref.
		units / mmol / mmol	(mM)	(mM)	β-5	β-O-4 (α-OMe)	β-O-4 (α-OH/OAc)	β-β	(%)	
H_2O_2/HRP	10-% aq. acetone, pH 3	2150 U / 4.8 / 2.4	10	5	**24**	xx	**16**	**12**	12	[80]
$Mn(OAc)_2$	glacial acetic acid	x / 4.8 / 2.4	10	5	22	xx	50 (□-OAc)	2	15	[80]
H_2O_2/HRP	10-% aq. methanol, pH 3 [a]	2150 U / 4.8 / 2.4	10	5	17	19	nm	nm	nm	Paper I
H_2O_2/HRP	90-% aq. methanol, pH 3 [a]	2150 U / 4.8 / 2.4	10	5	20	**42**	nm	nm	nm	Paper I
H_2O_2/HRP	10-% aq. methanol, pH 6 [a]	2150 U / 4.8 / 2.4	10	5	11	20	nm	nm	nm	Paper I
H_2O_2/HRP	70-% aq. dioxane, pH 3 [a]	2150 U / 4.8 / 2.4	10	5	23	xx	nm	nm	nm	see exp.
H_2O_2/HRP	90-% aq. methanol, pH 3 [b]	2150 U / 4.8 / 2.4	10	5	19	**36**	nm	8	nm	see exp.
Ag_2O	dry acetone	x / 0.6 / 1.5 eq. (10 h)	185	x	**44**	xx	non	5	8	[79]
Ag_2O	dry acetone + 1 M HCl (quenched)	x / 0.6 / 1.5 eq. (10 h)	185	x	**47**	xx	**32**	5	traces	[79]
Ag_2O	1:2 acetone-buffer pH 2.5 (3.1)	x / 0.6 / 1.5 eq. (4 h)	185	x	**24**	xx	**18**	**14**	non	[79]
Ag_2O	1:2 acetone-buffer pH 4.3 (5.3)	x / 0.6 / 1.5 eq. (5 h)	185	x	24	xx	traces	13	29	[79]
Ag_2O	1:1 acetone- water (pH 7.4)	x / 0.6 / 1.5 eq. (0.5 h)	185	x	17	xx	traces	10	36	[79]
Ag_2O	1:1 CH_2Cl_2-water (approx. pH 8)	x / 0.6 / 1.5 eq. (1 h)	185	x	12	xx	traces	25	20	[79]
H_2O_2/HRP	20-% aq. acetone, pH 7 (10 min)	60 U / 2.8 / 1.3	56	nm	12	xx	4	8	nm	[174]
H_2O_2/HRP	20-% aq. acetone, pH 7 (40 min)	20 U / 2.8 / 0.9	56	nm	17	xx	16	17	trace	[174]
H_2O_2/HRP	20-% aq. diglyme, pH 5	20 U / 2.8 / 1.9	56	nm	11	xx	4	8	15 (DHP)	[54]
H_2O_2/HRP	buffer solution, pH 7.4	? / 5.5 / 2.7 (?)	55	27	**48**	xx	**26**	24	nm	[47]
laccase I	20-% aq. acetone, pH 7 (1.5 hrs)	5500 U / 2.8 / x	140	x	18	xx	3	5	trace	[174]
laccase II	20-% aq. acetone, pH 7 (1.5 hrs)	135000 U / 2.8 /x	140	x	22	xx	non	9	non	[174]
H_2O_2/HRP	19-% aq. acetone, pH 4.5	1500 U / 0.6 / 0.6	156	nm	43	xx	7	nm	nm	[187]

4.2 Formation of spirodienones by oxidative coupling of methyl sinapate (Papers II and III)

The results reported in the previous sections have shown that methanol reacts very easily with quinone methide intermediates (Paper I). Therefore, methyl sinapate (**15**) was oxidazed using a HRP/H_2O_2 system in a methanol-buffer solution (0.02 M citrate-phosphate buffer, pH 4) with 30-% (v/v) methanol. The same reaction was performed in acetone-buffer solution (0.02 M, pH 4) with 20-% acetone. The only difference was the type of solvent, but the result was surprisingly different. The reaction scheme and the results are presented in Figure 24. Neudorffer et al. [193] obtained very similar results using the electrochemical oxidative coupling of 3,5-disubstituted 4-hydroxycinnamic ester derivatives in dry acetonitrile followed by treatment with a 0.5 M citrate-buffered aqueous solution of pH 6, and then by separation using silica gel chromatography. This technique yielded 20-% of a similar spirodienone product (**19**) as the spirodienone product (**16**) in the present study (Paper II). They used 3,5-di-*t*-butyl substituted 4-hydroxycinnamic acid ester as the starting material similar to the 3,5-dimethoxy substituted methyl sinapate (**15**) used in this study (Paper II). When they used methyl sinapate (**15**) as the starting material they obtained exactly the same product (**17**), even with the same 42-% yield as compared to our 41-% yield. Very bulky *t*-butyl groups have been observed to reduce the reactivity of nucleophiles towards quinone methides [79], and this might be the reason for a different kind of spirodienone structure with a double bond in the five membered ring. The acidic β-proton is removed faster than the nucleophilic water attacks the quinone methide intermediate. Wallis et al. [194] obtained a tetralol-type of dimer (**21**) in 61-% yield when methyl sinapate was oxidized by ferric chloride in aq. acetone. Water was able to act as a better nucleophile because of rather acidic conditions. Spirodienone-like structures were not observed.

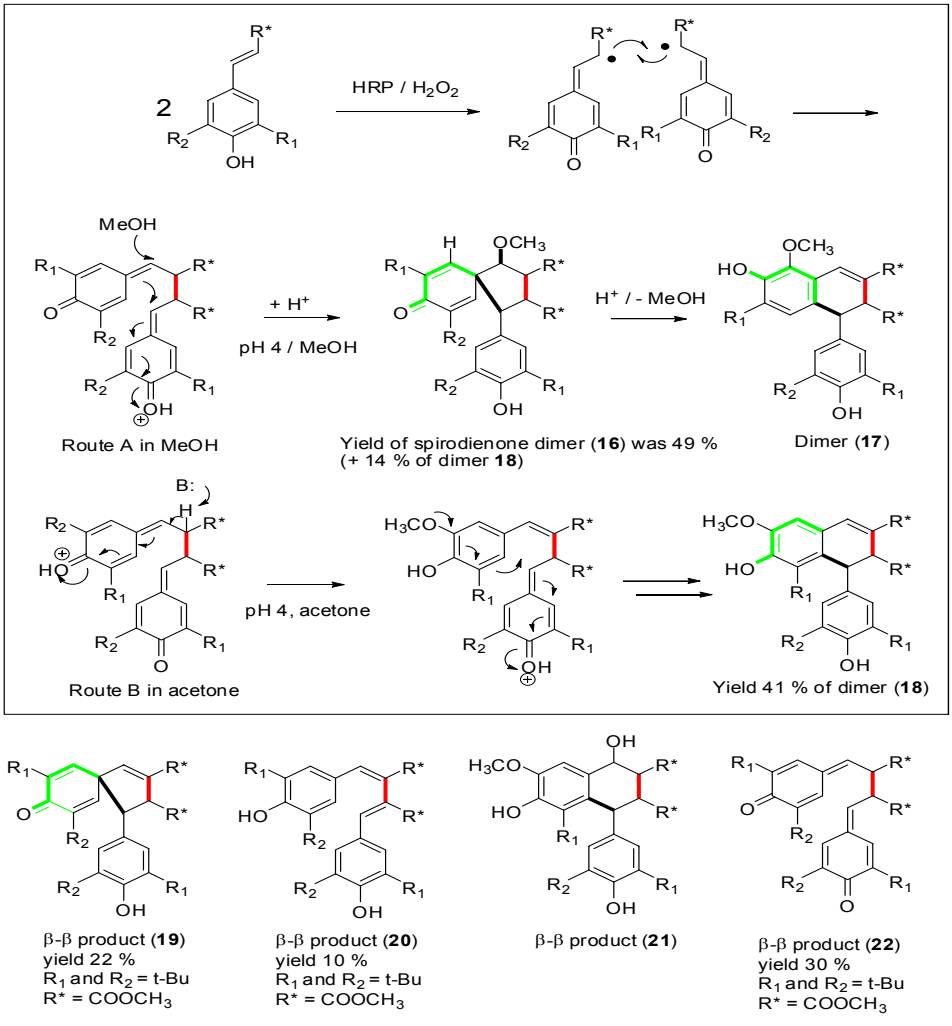


Figure 24. Dimerization of 3,5-disubstituted 4-hydroxycinnamics to β-β coupling ***(red bonds)*** *products:* ***The black, bolded bonds*** *have formed after the intramolecular attack of a nucleophile to the quinone methide intermediate forming either spirodienone or dihydronaphthalene structures, depending greatly on reaction conditions and substituents in the starting compound. R* = COOMe/Et or -CH3, R1 and/or R2 = -H, -OCH3 or -t-Bu. Green bonds illustrate the rearrangement and the difference between the products (**17**) and (**18**).*

Zanarotti et al. [76] used 5-methoxy isoeugenol as a starting material in dry organic solvents and Ag_2O as an oxidant. When they added a nucleophile after the formation of the quinone methide intermediate, β-O-4 dimers were obtained in rather good yields from 45 up to 95-% depending on the nature of the nucleophile used. Other dimeric products were not studied. Wallis et al. [81] obtained 56-% of the β-O-4 (α-OH) dimer of 5-methoxyisoeugenol, 2-% aryldihydronapthalene (**18**, R* = -CH_3), and 9-% tetralol dimers (**21**, R* = -CH_3) using 55-% aqueous acetone and $FeCl_3$ as an oxidant.

The spirodienone structure (R_2 = -H) similar to the compound (**16**) presented in Figure 20 was obtained tentatively by the oxidative dimerization of methyl ferulate (**2**) when performed in aq. methanol at pH 3 at a 16-% yield. See experimental data in Section 6.1.

4.3 Effect of catalysts (previously unpublished results)

Some results not previously published will be presented here. Different kinds of peroxidases or other oxidoreductases or inorganic single-electron oxidants can be used to generate phenoxy radicals. In this study four peroxidases – horseradish peroxidase (HRP), manganese-dependent peroxidase (MnP), lactoperoxidase (LPO) and lignin peroxidase (LiP) – were tested. Silver (I) oxide and tetraphenylporhyrinatomanganese(III) [(Mn(III)TPP] acetate or chloride were also used as oxidant systems, and iodosylbenzene or hydrogen peroxide were used as oxidants with Mn(III)TPP, but those results are presented and discussed in Section 4.1.1.

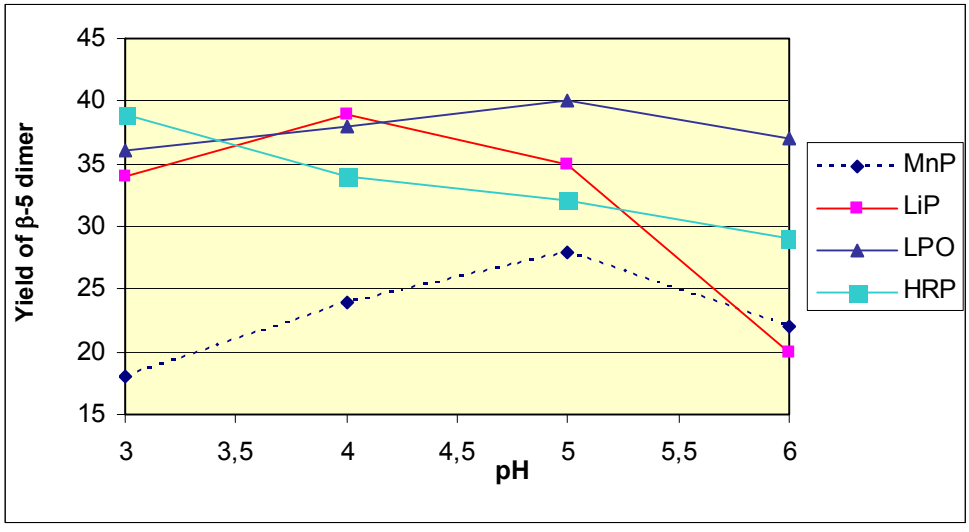


*Figure 25. Effect of four peroxidases on the yield of β-5 dimer (**5**) of methyl ferulate were tested. The conversions were always 100% when the reaction was catalyzed by LPO and HRP.*

The yield of the β-5 dimer (**5**) of methyl ferulate was almost the same and ca. 30–40-% in 10-% aq. methanol at pH 3 when LiP, LPO, or HRP were used as catalysts. The yield of dimer (**5**) seemed to be rather independent of the kind of catalyst used at pH 3 with the exception of MnP. The yield of β-5 dimer decreased as a function of increasing pH with LiP and HRP, but it was rather constant in the pH range of 3–6 with LPO. This may be due to the structure of the active centre of lactoperoxidase. The heme pocket of LPO has been reported to be more constrained than that of HRP [195], and the coupling of two phenoxyradicals may not be affected so much by the pH of a reaction medium. The yield of dimer (**5**) increased up to 28-% at pH 5 with MnP. MnP was not so efficient and its activity seemed to be lowest at pH 3 where the conversion was also low, i.e. only 36-%. The highest conversion 54-% was obtained at pH 5 with Mn-dependent peroxidase. So this enzyme may have a local activity optimum around pH 5 for the dehydrodimerization reaction to β-5-type dimer of methyl ferulate. LiP seemed to have a pH optimum around pH 4 where the conversion was 94-%. At other pH values the conversion was always lower and decreased quite fast to 43-% at pH 6. The conversions were always 100-% when the reaction was catalyzed by LPO and HRP. These results presented in Figure 25 show that the pH effect is not only a result of the common pH of the reaction

media but also the enzyme might play a very important role as a controlling substance in the oxidative coupling of two phenoxy radicals. This phenomenon has also been observed elsewhere.

The rate constant of LPO has been reported to be much less affected by organic solvents than that of HRP. [196] LPO has a comparatively compact heme pocket which may be the reason for its different behaviour. [195] This was observed in this work also when lactoperoxidase was used as a catalyst in 10-% aq. methanol at pH 6. The yield of β-5 dimer was still 37% while it was only 29-% when HRP was used in the same conditions. The yield of β-5 dimer (**5**) decreased from 39% at pH 3 to 29% at pH 6 when HRP was used as a catalyst.

4.4 Cross-coupling studies (Paper V)

When two different monolignols react in the oxidative coupling reaction (dehydrodimerization), the so-called cross-coupling reaction will occur. Two starting materials were choosen for these experiments: methyl sinapate MeSA (**15**) and 1-(4-hydroxy-3,5-dimethoxyphenyl)ethanol (**23**). Methyl sinapate and the compound (**23**) do not form any β-5, 5-5', or 5-O-4' coupling products together or independently. MeSA can only react in the β-position or also theoretically in the 1-position of the aromatic ring but the β-position is favored leading to β-β or β-O-4 products. 1-(4-hydroxy-3,5-dimethoxyphenyl)ethanol can react in the 1-position with the 1-hydroxyethyl substituent, or it can form the β-O-4 coupling product with methyl sinapate.

The reaction was performed in 30-% aq. acetone (0.02 M citrate-phosphate buffer, pH 3.5) by using HRP as a catalyst and H_2O_2 as an oxidant. Equimolar amounts of compounds (**15**) and (**23**) were reacted yielding two dimers identified after acetylation and separation using preparative liquid chromatography. The reaction scheme is presented in Figure 26. The dimer (**24**) with a spirodienone structure (β-1/α-O-α) was obtained in a rather good 19-% yield considering that 50-% of the starting material (**23**) did not react. The aryltetralin dimer (**18**) was obtained only in ca. 4-% yield.

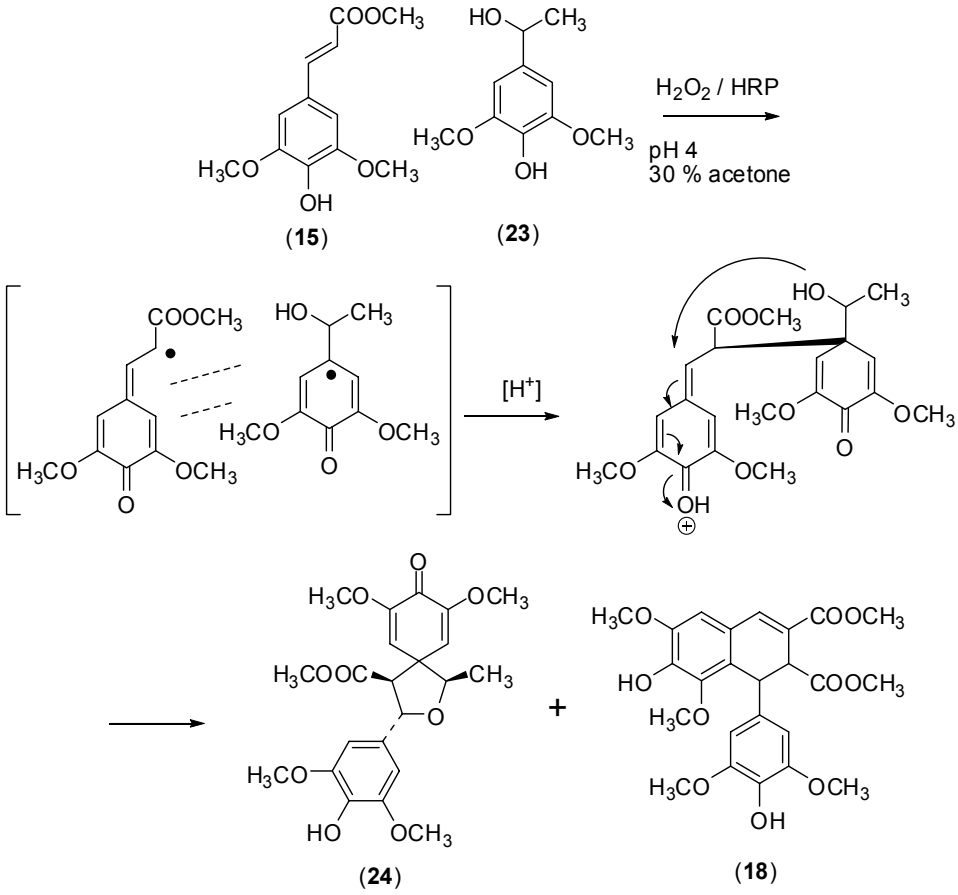


*Figure 26. Cross-coupling of methyl sinapate (**15**) and 1-(3,5-dimethoxy-4-hydroxyphenyl)ethanol (**23**) yielding 19-% of the dimer (**24**) with a spirodienone structure and a small amount (4-%) of the dimer (**18**) from the coupling of two methyl sinapate radicals.*

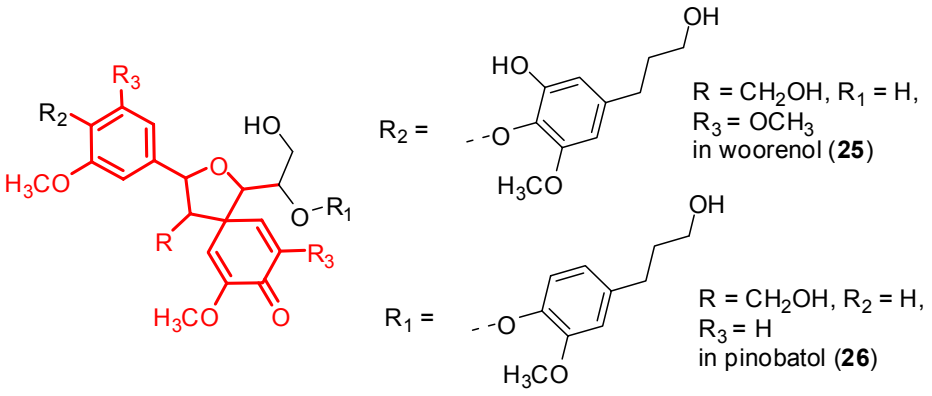


*Figure 27. Spirodienone lignan woorenol (**25**) was extracted from the rhizomes of Coptis japonica [197], and pinobatol (**26**) from pine bark (*Pinus sylvestris *L.). [18] The spirodienone skeleton (**24**) (red coloured) (R_3 =-- OCH_3, R_2 = -OH and R = -$COOCH_3$) was synthesized (Paper V).*

The spirodienone product (**24**) obtained here is the first synthetic spirodienone model compound formed by the so-called β-1 cross-coupling reaction (Paper V). [57] This finding together with other similar natural compounds found in plants such as woorenol (**25**) [197] and pinobatol (**26**) [18] (see Figure 27) which have the same kind of spirodienone structure, and with the earlier observations from wood analyses, these offer a new possible explanation and/or pathway to the existence of the β-1 structural units of lignins. The abundance of the β-1 structure has been estimated to range from 1-% to 15-% in spruce lignin. [198–200] The β-1 structures were earlier characterized mainly as 1,2-diarylpropane-1,3-diols (see compound **28** in Figure 28) obtained after either mild acidic hydrolysis of wood (spruce and beech) in dioxane-water [201, 202], or after acid hydrolysis [203], and according to the results from thioacidolysis/Raney-Ni degradative analysis [204], and from the DFRC method. [205] However, NMR spectroscopic observations have indicated that the 1,2-diarylpropane structure is only a minor component in lignin [146, 198, 206, 207], as also the ozonation study results have shown. [199] After those findings, the spirodienone structure has been proposed as a logical intermediate formed through a β-1 cross-coupling mechanism during lignin (bio)synthesis. [24, 148, 208, 209] The spirodienone structure has been observed as one of the important structures present in spruce and aspen lignins, with an abundance as high as 1.5–3-% in spruce lignin and about 1.8-% in aspen lignin. [210]

Spirodienone structures have also been observed to exist in the lignins of other plants such as kiwi, pear, and rhubarb. [134] These observations also indicate that the spirodinenone structure might be a fundamental structural unit in the lignins in all kinds of plants (see Table 7).

Table 7. Relative amounts of structural units in some fruit lignins. guaiacyl (G); syringyl (S); β-O-4 aryl ethers; phenylcoumaran (β-5); β-β units; dibenzodioxocin (DiB); spirodienones (Sp) and "traditional" β-1 units (F); and others are cinnamyl alcohol and arylglycerol end groups. The amounts of these structural units in acetylated mill wood lignin samples have been determined by NMR. [134]

Sample	%G	%S	% β-O-4	% β-5	% β-β	% DiB	% Sp+F	% Others
Pear	45,1	54,9	77,9	4,5	10,6	2,1	2,3	2,6
Kiwi	93,6	6,4	68,3	12,0	3,9	8,2	2,6	4,9
Rhubarb	3,6	96,4	93,0	-	5,8	-	1,3	-

Two possible mechanisms for the formation of isochroman structures (**27**) or β-1 structures (**28**, 1,2-diarylpropane-1,3-diols, R = -CH_2OH) from cyclohexadienone spiro compounds like the dimer (**24**) are presented in Figure 28. The synthesized spirodienone (Paper V) has a similar structure characterized in lignins. [209, 210] The traditional β-1 coupling reaction was also performed by using mild acidic hydrolysis in methanol to give the compound (**27**, R = -CH_2OH, R_2 = -OH, R_3 = -OCH_3) and acetaldehyde (not measured), Paper V. An alternative pathway to isochroman structures are suggested also to be possible by Ralph et al. [208] and Peng et al. [211]

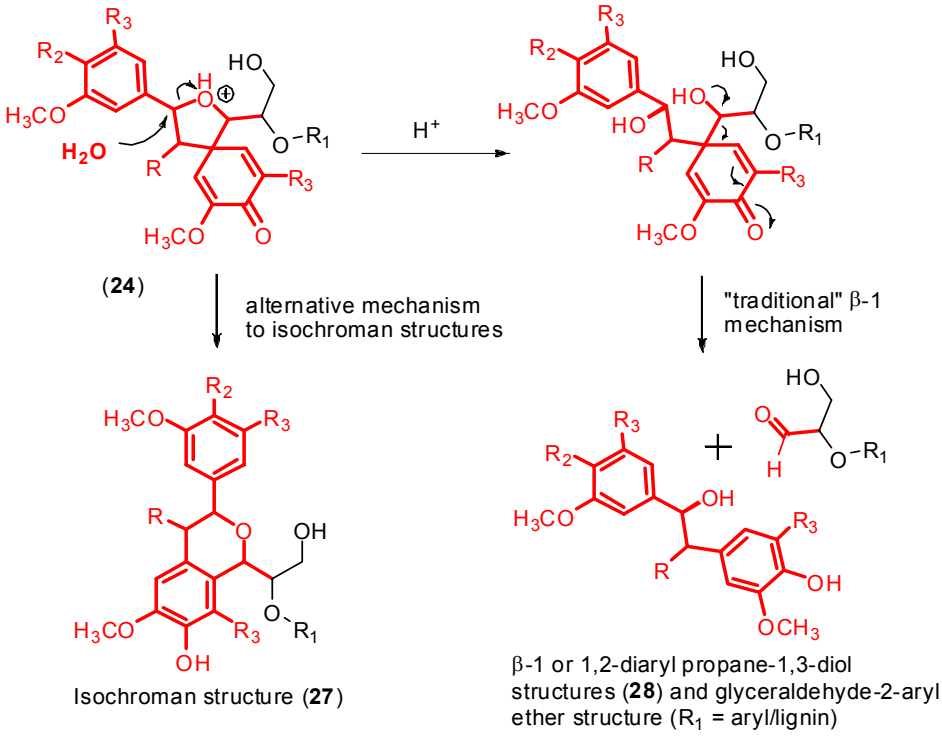


*Figure 28. Two possible mechanisms for the formation of isochroman structures (**27**) or β-1 structures (**28**, 1,2-diarylpropane-1,3-diols, R = -CH_2OH) from cyclohexadienone spiro compounds like the dimer (**24**) synthetized here (Paper V) or similar structures characterized in lignins. [209, 210]*

The proposed mechanism of transformation of the spirodienone structure (**24**) to isochroman (**27**) [211], and the very similar formation of the product (**17**) from the spirodienone dimer (**16**) is presented in Figure 29 (see Paper II). The C-C bond between the C-atom in the tetrahydrofuran ring and spiro carbon proposed to migrate via a "methoxymethyl cation" is very similar to that suggested for the formation of the aryltetralin structure (**17**) from the spirodienone dimer (**16**) of methyl sinapate. The migrating carbon in this so-called spirodienone-phenol rearrangement was able to carry a positive charge because of the resonance-stabilisation of the methoxy or alkoxymethylene group. [212, 213]

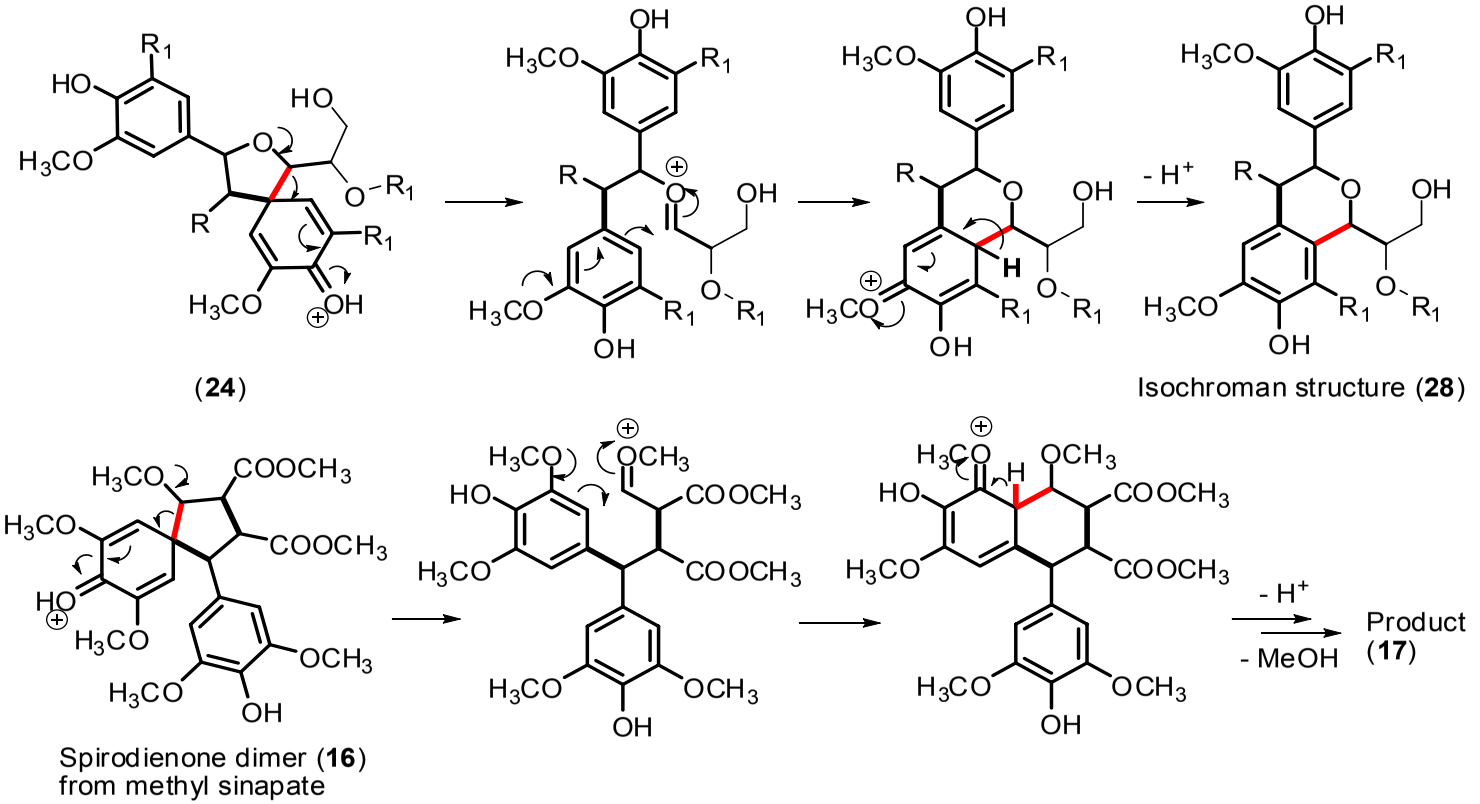


*Figure 29. Proposed mechanism for the transformation of the spirodienone structure (**24**) to isochroman (**28**) [211], and the very similar formation of the product (**17**) from the spirodienone dimer (**16**) (see Paper II). R_1 = '-H, -OCH_3 or the aryl/lignin.*

4.5 Preparation of enantiopure lignans (Papers IV and VI)

Natural lignans often exist in enantiopure forms. In order to determine which of the lignan enantiomers is bioactive and which may be, for example, a potential pharmaceutical pure enantiomers are often needed. Optically active, pure enantiomers of lignans and monolignols can be isolated and purified from plant material [125] or prepared by using enantioselective synthesis (Paper IV). [214] Pure enantiomers can be obtained by kinetic resolution [124], or by chiral resolution, for example, using chiral liquid [125] or supercritical cryogenic chromatography (Paper VI).

4.5.1 Stereoselective synthesis of enantiopure lignans and lignin model compounds (Paper IV)

Asymmetric synthesis is a powerful method for the preparation of enantiopure lignans and many synthetic strategies have been proposed and published. Chiral auxiliary substituents in starting compounds are used in all these methods to induce enantioselectivity. These synthetic methods need several steps and rather "hard" chemistry and reagents, and the overall yields after many steps are often only reasonable. The stereoselective synthesis of neolignans has been reviewed, for example, by Sefkow [214] and Ward. [112, 215]

More green and environmentally sustainable biomimetic methodologies have also been published recently. Boguchi et al. [216] were the first to perform the oxidative coupling of phenols in an stereoselective manner by using a methyl (R)-mandelyl substituent as a chiral auxiliary in sinapic acid. They used $FeCl_3$ as an oxidant in 2:1 organic solvent-water mixtures and THF, AcOH, IPA, CH_3CN, or acetone as cosolvents. They obtained 53-% yield of the major 1,2-*trans* diastereomer and 23-% of the minor 1,2-*trans* diastereomer, and also 8-% of the 1,2-*cis* diastereomer in acetone-water at 25 ^{o}C when the reaction time was 30 min. The total yield of aryltetralin-like structures was 84-%. They observed also that the reaction seemed to be much slower and not so diastereoselective in THF-water as in the other solvent systems.

The first biomimetic enantioselective oxidative coupling of monolignols using HRP/H_2O_2 catalyst/oxidant system in the coupling of a ferulic acid amide having

a ethyl (S)-alanine as a chiral auxialiary substituent was published in 1998 (Paper IV), (see Figure 30). The β-5 dimer of ferulic acid amide (**29**) was obtained in 70-% yield. The diastereomeric excess was observed to be 65-%. The main diastereomer (**30**) was reduced to optically pure dehydrodiconiferyl alcohol (**31**) using $LiBH_4$. This compound was shown to have the *2S,3R*-configuration by chiral chromatography, by authentic specimens of both enantiomers, and by the results and analytical methods published elsewhere. [217] This biomimetic synthetic procedure was used later in several studies yielding similar results by using other chiral auxiliary substituents presented in Table 8. The highest enantiomeric excess of a β-5 dimer, up to 84-%, was obtained when a very bulky chiral auxiliary substituent, Oppolzer's (+)-2,10-camphor sultam, was used. The result was in practice independent of the oxidant or solvent system used. The temperature in a range from -25 to +25 °C was not observed to have any remarkable effect on the enantioselectivity. [218]

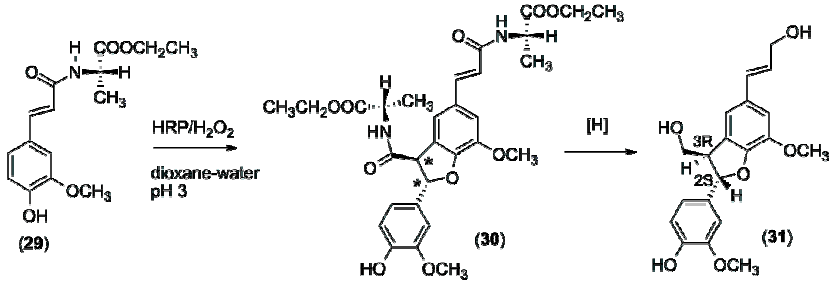


*Figure 30. Enantioselective synthesis of dehydrodiconiferyl alcohol (**31**) using ethyl-(S)-alaninate (**29**) as a chiral auxiliary substituent (Paper IV). The absolute configuration of (**31**) was determined by chiral chromatography according to the method of Hirai et al. [217]*

These results show that the enantioselective oxidative coupling of monolignols is possible using starting materials, monolignols, with chiral auxiliary substituents. The role of peroxidase was not discussed in the articles reviewed in Table 8. It is still possible that even HRP itself can also have some kind of role in the stereocontrol of dehydrodimerization of monolignols with chiral auxialiary substituents. It has been shown that HRP can, for example, catalyze sulfoxidation in an enantioselective manner. Enantiomeric excess was up to 68-%. [111] Biomimetic syntheses of some benzodioxane lignans from caffeic acid using HRP have been reported to be slightly enantioselective. [219] These

observations might indicate that also HRP may have some kind of control or role in the enantioselectivity obtained in the oxidative coupling reaction of monolignols with chiral auxiliary substituents. [111, 219]

Table 8. Enantioselectivities in some selected biomimetic oxidative coupling reactions of monolignols with chiral auxiliary substituents. E = enzyme, O = oxidant.

E/O	**Solvent system**	**Chiral auxiliary substitient**	**Yield (%)**	**e.e. (%)**	**Ref.**
HRP	30-% aq. dioxane, pH 3	Methyl (S)-alaninate	70	65	Paper V
$FeCl_3$	67-% aq. acetone	Methyl (R)-mandelyl	76	67	[216]
HRP	75-% aq. dioxane, pH 3.5	(S or R)-2-benzyloxazolidinone	40–50	21	[110, 218]
Ag_2O	CH_2Cl_2	(S or R)-2-benzyloxazolidinone	40–50	18–20	[110, 218]
HRP	75-% aq. acetone, pH 3	(S or R)-2-phenyloxazolidinone	40–50	59–62	[110, 218]
Ag_2O	CH_2Cl_2	(S or R)-2-phenyloxazolidinone	40–50	53	[110, 218]
HRP	75-% aq. acetone, pH 3.5	(+)-2,10-camphor sultam	40–50	81	[218]
Ag_2O	CH_2Cl_2	(+)-2,10-camphor sultam	40–50	80–84	[218]
HRP	30-% aq. dioxane, pH 3.5	Ethyl (S)-alaninate	70	65	[220]
HRP	30-% aq. dioxane, pH 3.5	(+)-2,10-camphor sultam	40	81	[220]
Ag_2O	CH_2Cl_2 (- 20 °C)	(+)-2,10-camphor sultam	40	80	[220]
Ag_2O	CH_2Cl_2 (+ 25 °C)	(+)-2,10-camphor sultam	35	84	[220]
HRP	75-% aq. dioxane, pH 4	(S)-phenylalanine ethyl ester	60	50	[121]
HRP	75-% aq. dioxane, pH 4	(S)-methylbenzylamine	50	40	[121]
HRP	75-% aq. dioxane, pH 4	(S)-2-phenyloxazolidinone	40	70	[121]

4.5.2 Preparative chiral chromatography as a potential method for obtaining enantiopure lignans and dilignols (Paper VI)

Several natural and synthesized lignans such as the methyl ester of dehydrodiferulic acid (diFA) and 3',4-di-*O*-methylcedrusin have been purified using chiral liquid chromatography as reported by Lemière et al. [125] They used several chiral columns such as a Chiralcel OD column for which they determined separation factors of 1.05 with EtOH/hexane (1:1) and 1.05 with 100-% ethanol as eluents. The best separation factor was obtained using Chiralcel OJ column (α = 1.26) with ethanol as eluent. The separation was performed in a preparative scale (2.1 g sample/2 kg chiral phase).

In this work (Paper VI) the method was tested for the chiral purification of diFA methyl ester using semipreparative column (10 x 250 mm) with 10 g chiral phase (Daicel Chiralcel OD) and hexane–2-propanol as the eluent (see Chapter 6, experimental). The best results were obtained using hexane–2-propanol in a 40:60 mixture with a flow rate of 0.5 ml/min and loading 0.325 g sample/kg phase with 0.05 ml injection volume. This was a similar loading of a monolignol as published earlier by Lemiere et al. [125] for the chiral separation of the same compound, the methyl ester of diFA (**5**, Figure 18, p. 47). The calculated productivity was 33 g/kg of phase per day with the separation factor 1.17 (resolution was 0.7) according to the results published in Paper VI. Therefore, the productivity with this size of a column would have been ca. 0.16 g of each enantiomer per day.

Wolf and Pirkle [221] have observed that low temperatures generally favour enantiomeric selectivity in syntheses as well as in separations. A new, promising and more powerful method for the chiral resolution of racemic mixtures for preparative scale production of lignans was developed by using pressurized/ supercritical carbon dioxide with a cosolvent such as ethanol or methanol at cryogenic temperatures (Paper VI). The method was used successfully for chiral separation of some interesting molecules such as the β-5 dimer (**5**) of methyl ferulate and enterolactone (**33**, Figure 32). A systematic approach was presented to find the optimum conditions for maximum throughput. Two chromatography columns were screened: Chiralcel OD CSP and Kromasil CHI-TBB using analytical scale columns (4.6 x 250 mm). The typical chromatograms are presented in Figure 31 with these columns under three different chromatographical conditions.

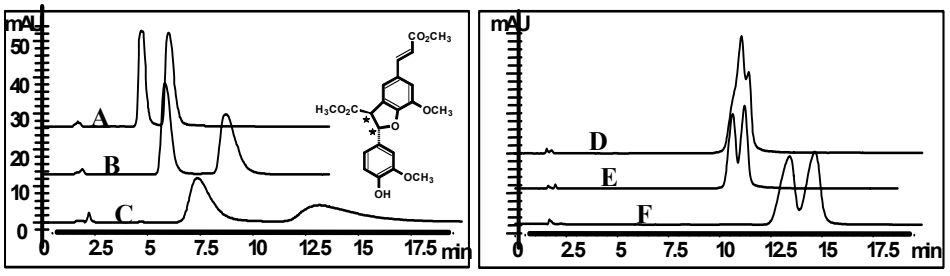


*Figure 31. Chiral separation of the β-5 dimer (**5**) of methyl ferulate using two different chiral phases and columns under three different chromatographic conditions A (+25 °C), B (+0 °C), C (–30 °C) with mobile phase CO_2/EtOH 20-% and with the column Chiralcel OD CSP, and D (+25 °C), E (+0 °C), and F (–25 °C) with mobile phase CO_2/EtOH 3-% and with the column CHI-TBB.*

With the optimization procedure presented in Paper VI it was possible to produce even 4.2 g of each pure enantiomer of the β-5 dimer (**5**) of methyl ferulate by using a semi-preparative size column (10 x 250 cm) of Chiralcel OD with the measured optimum productivity of 840 g/kg of phase/day. This is 25 times more than by using chiral liquid chromatography at room temperature.

(+/-)-Enterolactone was prepared in a 100 gram scale by using a four step synthetic route with an overall yield of 56-% [117, 118, 222, 223], see Figure 32. An efficient preparative liquid chromatographical method was developed and used successfully for the purification of enterolactone as well as its synthetic precursors and intermediates (see Figure 33). (+/-)-Enterolactone was resolved to pure enantiomers by using preparative supercritical fluid chromatography. This method was very efficient, productive, and fast.

The racemic mixture of enterolactone can be used, for instance, for bioactivity studies as those published elsewhere. [224, 225] The pure enantiomers of enterolactone were separated using the same methodology as presented in the case of dehydrodiferulate (**5**) with the Chiralcel OD column. 128 mg of (-)-enterolactone (peak A) and 118 mg of (+)-enterolactone (peak B) were prepared rather easily (see Figure 34).

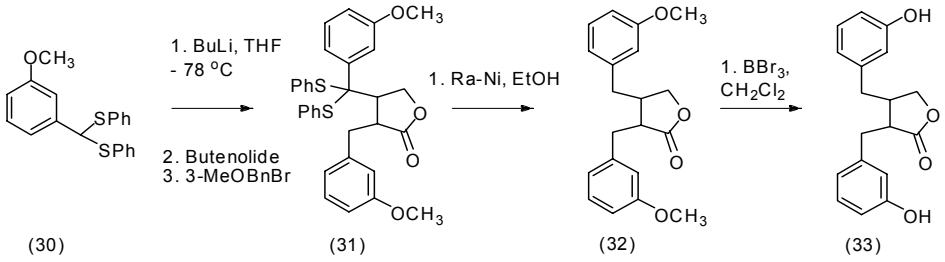


Figure 32. Synthesis scheme for the preparation of racemic enterolactone (see Chapter 6, Experimental)

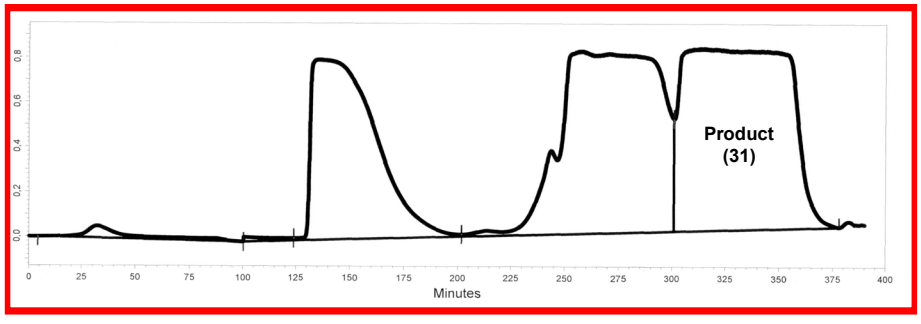


*Figure 33. Typical chromatogram showing preparative separation of the product from unreacted starting materials and by-products in the purification of diphenylthioacetal of di-O-methyl enterolactone (**31**). Ca. 40 g of a crude product mixture was dissolved into a suitable amount of ethyl acetate and loaded into a 70 x 460 mm Büchi glass column filled with flash silica gel. The last peak was the product (**31**).*

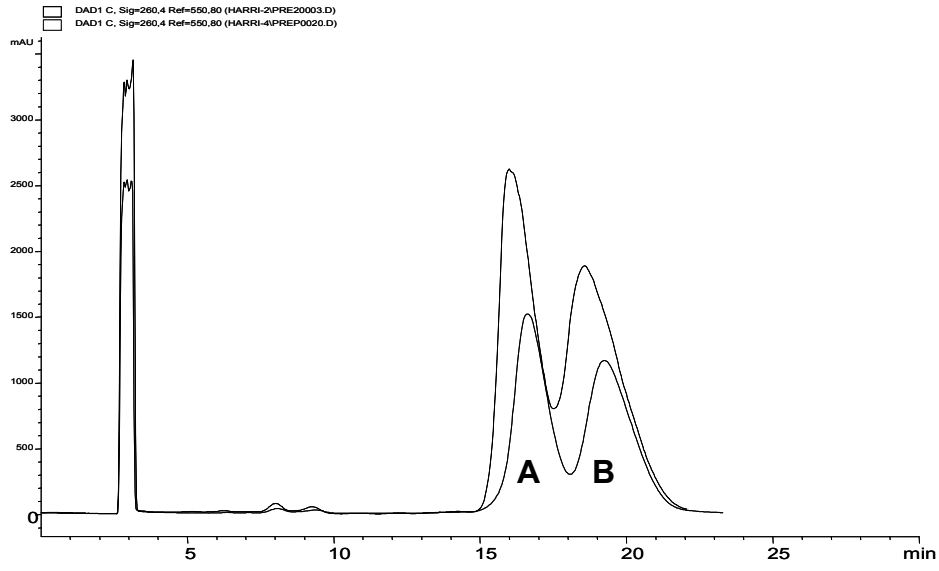


*Figure 34. Chromatogram showing the chiral separation of (+)- and (-)-enterolactone using preparative chiral SFC (Chiracel OD 10 x 250). 5–10 mg of enterolactone was injected as 100 mg/mL acetone solutions. The first peak (**A**, RT = 16 min) was determined to be (-)-enterolactone and the peak (**B**, RT = 19 min) was (+)-enterolactone (see experimental part, Chapter 6).*

These results show that cryogenic chiral chromatography may be a valuable method for purifying lignans as enantiomerically pure compounds at a preparative scale.

5. Conclusions

The results of this thesis show that the regio- and stereoselectivity of the oxidative coupling reaction of phenols are equally dependent both on stereoelectronic effects of the structures of starting materials and very much on the reaction conditions where the most important parameters are: 1) the catalyst and oxidant used and their concentrations in the reaction media, 2) the solvent system which can be water mixed with water miscible organic solvents or a hydrophobic (dry) organic solvent; 3) the pH of the reaction media, and 4) the concentration and type of nucleophilic species in the reaction media.

The reactions of the three 3-methoxy-4-hydroxycinnamics with different kinds of substitution at Cγ gave very different results. This shows that the stereoelectronic effects are important in the coupling reaction of phenoxy radicals to yield the primary coupling structures and the basis for the ratio of dimeric structures. Isoeugenol with an electron-releasing substituent -CH_3 gave a β-5 dimer at high yield and together with β-O-4-α-OCH_3 product even in 88-% yield in 90-% aq. methanol at pH 3. Methyl ferulate gave also a β-5 dimer in a rather high yield of up to 50-% in the same conditions. Coniferyl alcohol gave three main dimeric products in 75-% total yield in 70-% aq. methanol at pH 3, where methanol as a rather good nucleophile produced β-O-4-OCH_3 dimers even in 45-% yield.

Methyl sinapate was also dimerized in aq. methanol to determine the effect of methanol. Surprisingly a new kind of spirodienone structure was obtained whereas the aryltetralin structure was the main product in aq. acetone.

The spirodienone dimer was also obtained in a 19-% yield when a so-called cross-coupling reaction was performed with methyl sinapate and 1-(4-hydroxy-3,5-dimethoxyphenyl)ethanol. After this study was published, similar spirodienone structures have been found to exist commonly in many wood species and other plants, also. The spirodienone structures and their reactions leading to other structures in lignans and lignins seem to be rather common in nature.

The effect of catalysts such as different peroxidases and also inorganic catalysts were also studied. The results show that the peroxidases, more than the other

catalysts used, may have an important effect on the regioselectivity of the oxidative coupling reaction in the same manner as observed and published by several other research groups elsewhere.

The formation of the primary bond in the oxidative coupling of two phenoxy radicals forms the basis for the ratio of different possible dimeric structures. The stereoelectronic effects due to the structural differences in the monolignols are the most important factors, but the results show that the reaction medium also has a great influence on this ratio. The basic reason is not clear but one explanation may be the effect of reaction parameters such as pH and organic cosolvents on the substrate-enzyme interactions.

The results of the addition reactions of suitable nucleophiles were clearly dependent both on the stereoelectronic effects of the structure of the quinone methide intermediate, on the nucleophiles and their concentrations in the reaction medium, and on the other reaction parameters such as pH and solvent system. The other reaction steps following this step yielded many kinds of stable structures and end-products which were also dependent on the structure of this intermediate and on the reaction conditions. Methanol was found to react readily as a nucleophile with quinone methide intermediates.

For the purpose of exploring the possibilities to synthesize enantiopure lignans and lignin model compounds a stereoselective oxidative coupling reaction was also performed using a phenolic substrate with a chiral auxiliary substituent. The reaction proceeded in good yield and stereoselectivity. Another good method for obtaining enantiopure lignans was chiral chromatography, especially in cryogenic conditions with a carbon dioxide-methanol mixture as eluent. This chiral resolution method was validated for preparative resolution of racemic mixtures of dilignols and lignans.

6. Experimental

6.1 Synthesis of a spirodienone dimer of methyl ferulate

5.2 g (25.1 mmol) of methyl ferulate (**2**) was dimerized by using 100 mg of HRP (Sigma 250 U/mg) dissolved in 10 ml water and 12.6 mmol of H_2O_2 as an oxidant. Methyl ferulate was dissolved into 85 ml methanol and 125 ml buffered water solution was added (pH 3.5, 0.02 M citrate-phosphate buffer). HRP was added and followed by the addition of 16 ml H_2O_2 in 10 min into the reaction mixture. The reaction mixture was stirred for 2 hrs. The reaction mixture was filtered through 0.45 μm membrane filter. The solvents were evaporated to dryness. The reaction mixture was acetylated using an acetic anhydride-pyridine mixture (1:1) overnight at rt. The dimers were separated using a silica column and hexane-ethyl acetate as eluent. The yield of β-5 dimer was 43-% and the yield of the spirodienone dimer of methyl ferulate was tentatively 16-%. The ^{1}H-NMR spectra and the tentative signal assignments are presented in Table 9. A 200 MHz Varian NMR spectrometer was used. The solvent was $CDCl_3$. The spectrum is shown in Figure 35.

The peaks are assigned tentatively by comparing to the ^{1}H-NMR of spirodienone dimers of methyl sinapate (**16**), which are labelled to be the dimers **3a** and **3b** in Paper II. The peaks of the dimer **3a** from the five membered spiro ring were closer to the peaks of this possible spirodienone dimer of methyl ferulate.

Table 9. The ^{1}H-NMR (200 Mhz, CHcl3) spectral parameters and the tentative signal assignments of the synthesized spirodienone dimer of methyl ferulate.

Assignment	**δ_H (ppm)**	**mult.**	**protons**	***J* (Hz) (*)**
4'-$OCOCH_3$	2,27	s	3	no
4-OCH_3	3,37	s	3	no
7-OCH_3	3,53	s	3	no
3-CH	3,53	dd	1	10,7 and 6,4
3-$COOCH_3$	3,65	s	3	no
3'-OCH_3	3,74	s	3	no
2-$COOCH_3$	3,83	s	3	no
1,2 or 4-CH	3,87	m	1	nd
1,2 or 4-CH	3,96	m	1	nd
1,2 or 4-CH	4.03	m	1	nd
6-CH	5,66	d	1	2,6
9-CH	6,33	d	1	10,2
2'-CH	6,67	d	1	1,9
6'-CH	6,72	dd	1	2,0 and 8,2
5'-CH	6,89	d	1	8,1
10-CH	7,19	dd	1	2,6 and 10,3

(*) The couplig constants were determined by a first order interpretation and are approximate.

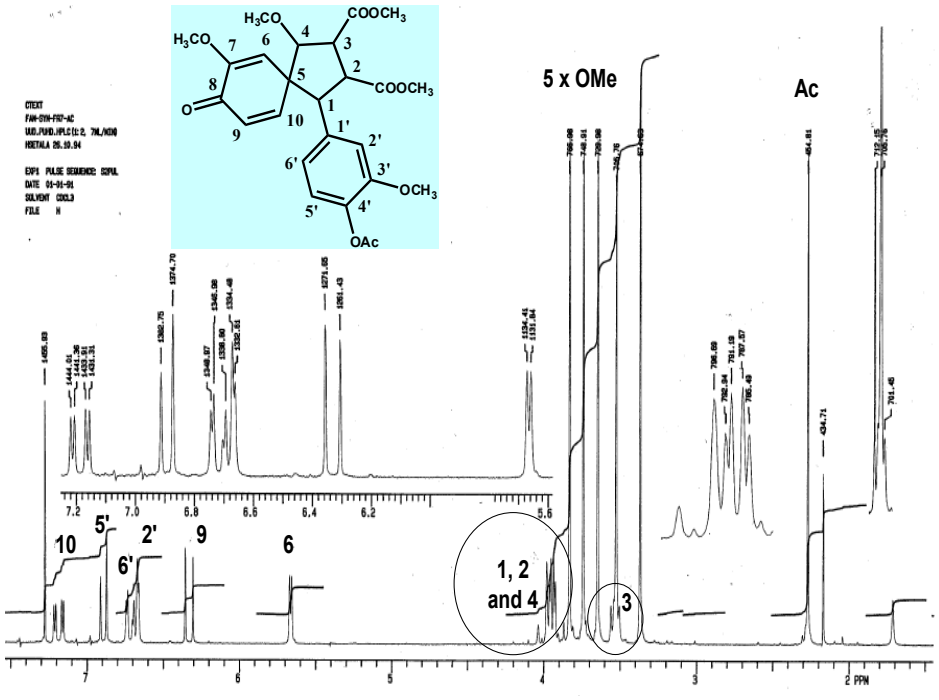


Figure 35. The [1]H-NMR spectrum of the supposed spirodienone dimer of methyl ferulate.

6.2 Dehydrodimerization experiments in dioxane

The small scale screening experiments were described in Paper I.

6.3 Dehydrodimerization of methyl ferulate using four different peroxidases

Lignin peroxidase (LiP) was obtained from the University of Helsinki (LiP was isolated from *Phlebia radiata*, reported activity 42 nkat/ml by NADH/veratryl alcohol method, solution in 0.1 M acetate buffer, pH 5). [226] Manganase-dependent peroxidase (MnP) was from VTT (reported activity 320 nkat/ml by NADH method/verartryl alcohol method, solution in 0.025 M succinate-lactate buffer, pH 4). Lactoperoxidase (LPO) was from Sigma (L2005, 80 U/mg, powder) and horseradish peroxidase (HRP) from Serva (31943, 250 U/mg, lyophilized salt-free powder). Before use, the activities of the used enzymes LiP, MnP, LPO, and HRP were determined by the purpurogallin method where the formation of oxidation product was measured by a UV-VIS spectrometer at wavelength 420 nm. [227] The activities were determined in 10-% aq. methanol, 0.1 M citrate-phospate buffer with pH 3–6. Total volume of the reaction mixture was 2 ml. 5 μmol methyl ferulate in 0.2 ml MeOH was added into 1.3 or 1.7 ml of a buffer solution. 0.1 or 0.5 ml (ca. 1 U measured by the pyrogallol method) of the enzyme was added. The reaction was started by adding 30 μl (2.5 μmmol) H_2O_2. The yield of β-5 dimers and conversions of the reaction were measured as described in Paper I by HPLC and using synthetized β-5 dimers as external standards. The results and some reaction parameters are shown in Table 10.

Table 10. The reaction conditions with four peroxidases, yields and conversions.

Peroxidase	**Enzyme solution**	**Buffer solution**	**pH 3[e] yield (co)**	**pH 4[e] yield (co)**	**pH 5[e] yield (co)**	**pH 6[e] yield (co)**
	(ml)	(ml)	(%)	(%)	(%)	(%)
MnP[a]	0,5	1,3	18 (36)	24 (47)	28 (52)	22 (42)
LiP[b]	0,1	1,7	34 (83)	39 (94)	35 (71)	20 (43)
LPO[c]	0,1	1.7	36 (100)	38 (100)	40 (100)	37 (100)
HRP[d]	0,1	1.7	39 (100)	34 (100)	32 (100)	29 (100)

a) in 0.025 M buffer, pH 4, the concentration of $MnSO_4$ in the added citrate-buffer solution was adjusted to 1 mM; b) in 0.1 M sodium acetate buffer, pH 5; c) dissolved in 0.01 M citrate-phosphate buffer, pH 6; d) dissolved in 0.005 M citrate-buffer, pH 6. e) The pH values of mixed solutions were not corrected. The pH value of added buffer solution was used. (co) = conversion %.

6.4 Synthesis of enterolactone and purification of its enantiomers by chiral resolution

Synthesis of enterolactone (33, Figure 32, p. 74**)**

Compound (**30**) was synthesized from 3-methoxybenzaldehyde and thiophenol with $AlCl_3$ in dry dichloromethane as described by van Oeveren 1994 et al. [118] The crude product was purified by flash chromatography with hexane-ethyl acetate as the eluent yielding 89-% pure compound (**30**) (lit. [118] 81-%). The thioacetal of di-O-methyl enterolactone (**31**) was prepared from compound (**30**) by reaction with n-hexyllithium and 2-butenolide in THF (–78 ^{o}C), followed by in situ alkylation with 2-methoxybenzylbromide in the presence of HMPA. This is also called "tandem" addition. The synthesis was slightly modified from the method published by Pelter et al. [222] The yield was 75-% (lit. [222] 65-%) after a preparative liquid chromatographic purification step (see the chromatogram in Figure 34). Treatment of compound (**31**) with Raney-nickel in refluxing ethanol gave di-O-methyl enterolactone (**32**) in approx. quantitative yield. [223] Demethylation of compound (**32**) with boron tribromide in CH_2Cl_2 (–20 ^{o}C) provided enterolactone by using the procedure published by Bode et al. [117] The crude product was purified by preparative liquid chromatography to yield 85-% enterolactone (**33**). See Figure 32. Enterolactone was also easilly reduced to enterodiol (**34**) using $LiAlH_4$ in refluxing THF. Enterodiol was crystallised from an ethyl acetate – ethanol mixture yielding 98-% of the white product.

Preparative liquid chromatography. The preparative HPLC with two preparative pumps (flow rate 0.1 to 300 ml/min), an injection pump (flow rate 0.1 to 10 ml/min), and UV-VIS detector controlled by the Shimadzu Class-VP automated software system were used as the chromatographic instrument. Büchi-685 glass columns (460 x 50 mm or 460 x 70 mm with pretreatment column) were used and self-packed with flash silica gel 60 (particle size 0.040, see Figure 32, 0.063 mm) by the wet/slurry filling method. The maximum input pressure was 40 bar. The flow rate was usually from 7 up to 15 ml/min when using hexane – ethyl acetate as the eluent. The gradient elution was used to ensure optimal resolution. The amount of loaded sample was dependent on the resolution and solubility but it was usually 10 to 50 grams when using a glass column of 460 x 70 mm filled with ca. 1 kg of silica gel. Elution times varied from 5 to 10 hours per one separation or injection. Injection/loading of a sample was performed by using an

injection pump or a Rheodyne loop injector (50 ml). An example of the resolution and purification of diphenyl thioacetal of di-O-methyl enterolactone (31) is shown in Figure 33 (p. 74).

Preparative supercritical fluid chromatography (SFC), (Figure 34, p. 75): 100 mg enterolactone was dissolved into 1 ml acetone. 10 mg (100 ml) enterolactone was injected into a Chiracel OD 10 x 250 (Chiral Technologies Inc) column. CO_2 with 16-% methanol was used as eluent. Temperature was +22 ^{o}C. The productivity was ca. 100 mg of each pure enantiomer of enterolactone in a day. The loading was 1 g of (+/-)- enterolactone per 1 kg CSP (dotted line) or 0.5 g per 1 kg of chiral solid phase (CSP). The first peak (**A**, RT = 16 min) was determined to be (-)-enterolactone and the second peak (**B**, RT = 19 min) was (+)-enterolactone. Characterization of the separated fractions (**A**) and (**B**) was performed by comparing to an authentic reference sample – a pure enantiomer of (-)-enterolactone – at the Department of Organic Chemistry, Åbo Akademi University in the manner published earlier by Saarinen et al. [228]

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Setälä, Harri

Title

Regio- and stereoselectivity of oxidative coupling reactions of phenols

Spirodienones as construction units in lignin

Abstract

Dimeric phenolic compounds – lignans and dilignols – form in the so-called oxidative coupling reaction of phenols. Enzymes such as peroxidases and laccases catalyze the reaction using hydrogen peroxide or oxygen, respectively, as oxidant generating phenoxy radicals which couple together according to certain rules. In this thesis, the effects of the structures of starting materials – monolignols – and the effects of reaction conditions such as pH and solvent system on this coupling mechanism and on its regio- and stereoselectivity have been studied.

After the primary coupling of two phenoxy radicals a very reactive quinone methide intermediate is formed. This intermediate reacts quickly with a suitable nucleophile which can be, for example, an intramolecular hydroxyl group or another nucleophile such as water, methanol, or a phenolic compound in the reaction system. This reaction is catalyzed by acids. After the nucleophilic addition to the quinone methide, other hydrolytic reactions, rearrangements, and elimination reactions occur, leading finally to stable dimeric structures called lignans or dilignols. Similar reactions occur also in the so-called lignification process when monolignol (or dilignol) reacts with the growing lignin polymer. New kinds of structures have been observed in this thesis. The dimeric compounds with a so-called spirodienone structure have been observed to form both in the dehydrodimerization of methyl sinapate and in the β-1-type cross-coupling reaction of two different monolignols. This β-1-type dilignol with a spirodienone structure was the first synthesized and published dilignol model compound, and at present, it has been observed to exist as a fundamental construction unit in lignins.

The enantioselectivity of the oxidative coupling reaction was also studied for obtaining enantiopure lignans and dilignols. A rather good enantioselectivity was obtained in the oxidative coupling reaction of two monolignols with chiral auxiliary substituents using peroxidase/H_2O_2 as an oxidation system. This observation was published as one of the first enantioselective oxidative coupling reaction of phenols. Pure enantiomers of lignans were also obtained by using chiral cryogenic chromatography as a chiral resolution technique. This technique was shown to be an alternative route to obtain enantiopure lignans or lignin model compounds in a preparative scale.

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Fenolien hapettavan kytkentäreaktion regio- ja stereoselektiivisyys
Spirodienonit ligniinin rakenneyksiköinä

Tiivistelmä

Dimeeriset lignaanit ja dilignolit muodostuvat ns. fenolien hapettavassa kytkentäreaktiossa, jossa fenolisista monolignoleista syntyvät fenoksiradikaalit kytkeytyvät toisiinsa tiettyjen lainalaisuuksien mukaisesti. Reaktiota katalysoivat entsyymit, kuten peroksidaasit ja lakkaasit, sopivan hapettimen – joko vetyperoksidin tai hapen – läsnä ollessa. Tässä väitöskirjassa käsitellään näiden kytkeytymisten seurauksena syntyvien primääristen rakenteiden ja sitä kautta syntyvien dimeeristen yhdisteiden syntymekanismeja ja niihin vaikuttavia tekijöitä, kuten sitä, mitkä lähtöaineen rakenteesta johtuvat stereoelektroniset syyt johtavat erilaisten dimeeristen rakenteiden syntyyn; ja mikä on reaktio-olosuhteiden vaikutus näiden rakenteiden syntyyn. Tässä väitöskirjassa on tutkittu kuuden erilaisen monolignolin rakenteen sekä liuotinsysteemin ja pH:n vaikutusta; ja myös jonkin verran katalyytin sekä hapettimen vaikutusta reaktioiden regio- ja stereoselektiivisyyteen.

Hapettavan kytkentäreaktion jälkeen tapahtuvat sekundääriset reaktiot, kuten nukleofiilinen additio kinonimetidivälituotteeseen ja sitä seuraavat erilaiset hydrolyyttiset reaktiot, toisiintumiset ja eliminoitumisreaktiot, johtavat lopulta stabiileisiin dimeerisiin rakenteisiin. Näihin reaktiovaiheisiin vaikuttavia tekijöitä on myös käsitelty tässä väitöskirjassa. Kinonimetidi on syntyvän kytkentäreaktion tuote, välituote, joka on hyvin reaktiivinen (vaikkakin voi olla tietyissä olosuhteissa melko pysyvä) ja reagoi nukleofiilien kanssa joko molekyylien välisissä reaktioissa (vesi, fenolinen tai alifaattinen hydroksyyliryhmä, tiolit yms.) tai molekyylin sisäisesti esim. tarjolla olevan hydroksyyliryhmän kanssa synnyttäen mm. erilaisia rengasrakenteita (furaanit, bentsofuraanit). Nämä rakenteet ovat melko pysyviä ja yleisiä eristetyissä lignaaneissa ja ligniineissä. Kuitenkin jotkin niistä voivat olla myös välituotteita muiden lignaanien muodostumisreitissä ja myös mahdollisia reittejä tiettyjen ligniinissä esiintyvien rakenneosien muodostumiselle. Eräs tällainen välituotetyyppi ovat ns. spirodienonirakenteiset yhdisteet, joita esiintyy luonnossa stabiileina rakenteina lignaaneissa ja ligniinissä. Spirodienonirakenteinen dimeeri kuitenkin reagoi melko helposti mm. happamissa olosuhteissa toisiintumalla eri rakenteeksi. Spirodienonirakenteet selittävät osaltaan ligniinien ns. β-1-rakenteiden syntymismekanismeja. Yleisesti ottaen varsinaisen hapettavan kytkentäreaktion jälkeiset sekundääriset reaktiot voivat olla hyvin monimutkaisia ja johtaa suureen määrään rakenteellisesti hyvin erilaisia dimeerejä – lignaaneja. Lähtöaineiden rakenteen ja reaktiota katalysoivan entsyymi-hapetinsysteemin lisäksi pH-vaikutus, liuotinsysteemi, muiden nukleofiilisten reagoivien aineiden vaikutus (nukleofiilisyys, konsentraatiot); ja intra- vs. intermolekulaarisen reaktion nopeus välituotteen stabiloitumisessa lopputuotteeksi ovat tärkeitä reaktioparametreja.

Polymeerisen ligniinimolekyylin syntyessä kytkeytymisreaktion lainalaisuudet ovat osin toisenlaisia, koska tässä reaktiotyypissä – polymeroitumisessa – kasvava ligniinimolekyyli reagoi monomeerisen (tai dimeerisen) fenolisen yhdisteen, monolignolin, kanssa. Vallitseva selitys lignifikaatiosta, ligniinin syntymisestä, perustuu teoriaan, jonka mukaan tietyistä käytettävissä olevista monomeerisista yhdisteistä, monolignoleista, syntyy tiettyjen kombinatoriaalisen kemian lainalaisuuksien mukaan erilaisia ligniinin perusrakenneosia ilman esimerkiksi entsyymin ohjaavaa vaikutusta. Syntyvien rakenteiden keskinäinen suhde ligniinissä perustuu pikemminkin reagoivien monolignolien rakenne-eroavaisuuksiin (hapetuspotentiaalit, stereoelektroniset tekijät), konsentraatioihin ja syöttönopeuteen ligniinipolymeerin kasvaessa hapettavassa kytkentäreaktiossa; sekä erilaisten reaktio-olosuhteiden vaikutukseen. Tässä väitöskirjatyössä syntetisoitu β-1-ristikytkentä-mekanismilla syntynyt dimeeri on laatuaan ensimmäinen kokeellisesti valmistettu spirodienonirakenteinen dilignoliyhdiste. Rakenteen on myöhemmin todennettu esiintyvän yleisesti yhtenä ligniinien perusrakenneosana. Väitöskirjassa on valmistettu myös muita spirodienoni-tyyppisiä dimeerejä.

Lisäksi väitöskirjassa on tutkittu monolignoliin liitetyn kiraalisen substituentin vaikutusta hapettavan kytkentäreaktion enantio-selektiivisyyteen. Menetelmällä pystyttiin valmistamaan dimeerisiä rakenteita hyvällä enantioselektiivisyydellä. Julkaisu on eräs ensimmäisistä maailmassa. Puhtaita enantiomeereja voidaan valmistaa myös käyttäen ns. kiraalisia resoluutiotekniikoita. Tässä työssä tutkittiin ns. kiraalisen kromatografian käyttöä puhtaiden enantiomeerien valmistamiseksi raseemisista lignaaneista.

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